SMARTPHONE-ASSISTED MICROSCOPIC IMAGING PREDICTION OF MASS AND CHLOROPHYLL CONTENTS IN DUCKWEEDS

HOW MUN CHENG

UNIVERSITI SAINS MALAYSIA

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by

HOW MUN CHENG

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ANGGARAN JISIM DAN KANDUNGAN KLOROFIL DALAM PELBAGAI JENIS DUCKWEED DENGAN PENGIMEJIAN MIKROSKOPI MEGGUNAKAN TELEFON PINTAR

ABSTRAK

Teknik analisis imej makroskopik boleh digunakan untuk mengukur pelbagai ciri fizikal tumbuhan dengan ketepatan yang baik, yang telah dibuktikan oleh pelbagai penyelidik dalam beberapa tahun kebelakangan ini. Memandangkan pengimejan mikroskopik yang membolehkan pemerhatian yang lebih terperinci terhadap struktur tumbuhan berbanding dengan pengimejan makroskopik, penyiasatan mengenai kemungkinan menganggarkan jisim dan kandungan klorofil tiga spesies duckweed yang berbeza, iaitu *Spirodela polyrhiza*, *Lemna minor* dan *Wolffia arrhiza* dengan telefon pintar menangkap imej mikroskopik telah dijalankan dalam kajian ini. Didapati bahawa untuk korelasi jisim dengan luas, pembesaran yang paling sesuai untuk *Spirodela polyrhiza*, *Lemna minor* dan *Wolffia arrhiza* masing-masing ialah 1.2x, 1.2x dan 1.5x. Keputusan menunjukkan korelasi yang baik antara jisim dan luas tumbuhan setiap spesies dengan nilai R^2 lebih tinggi daripada 0.9. Sebaliknya, pembesaran terbaik untuk korelasi kandungan klorofil dengan parameter warna dan kombinasi adalah sama bagi ketiga-tiga spesies duckweed iaitu 2.5x. Nilai R^2 yang agak rendah (< 0.7) diperolehi untuk korelasi kandungan klorofil dengan setiap parameter warna dan gabungan dengan model regresi linear. Model regresi bukan linear dengan pemodelan Artificial Neural Network (ANN) menunjukkan kesesuaian yang lebih baik daripada model regresi linear kerana nilai R^2 yang jauh lebih tinggi (> 0.8) diperolehi bagi setiap model yang dibangunkan. Model

R+G+B (R^2 = 0.9029; RMSE = 1.1506 mg/L) adalah model terbaik untuk meramalkan kandungan klorofil dalam *Spirodela polyrhiza* manakala model B adalah model terbaik untuk *Lemna minor* (R^2 = 0.9033; RMSE = 0.3375 mg/L) dan *Wolffia arrhiza* (R^2 = 0.9624; RMSE = 1.1180 mg/L). Jisim plantlet duckweed dapat diramal dengan baik dengan model regresi linear manakala ramalan kandungan klorofil dalam plantlet duckweed dapat dilakukan menggunakan model ANN dengan ketepatan yang tinggi.

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ABSTRACT

The macroscopic image analysis technique can be utilized to quantify various physical features of plants with good accuracy, which has been proven by various researchers in recent years. Since microscopic imaging allows a more detailed observation of the plant structures compared to macroscopic imaging, investigation on the possibility of estimating the mass and chlorophyll content of three different species of duckweeds, namely *Spirodela polyrhiza*, *Lemna minor and Wolffia arrhiza* with smartphone captured microscopic images were carried out in this study. The most suitable magnification for correlation of mass with area for *Spirodela polyrhiza*, *Lemna minor* and *Wolffia arrhiza* were 1.2x, 1.2x and 1.5x respectively. The results showed good correlation between mass and area of plantlet of each species with R^2 value of higher than 0.9 at the most suitable magnification. On the other hand, the best magnification for the correlation of chlorophyll content with color parameters and combinations was the same for all the three species which was 2.5x. Relatively low R^2 value (< 0.7) was obtained for the correlation of chlorophyll content with each color parameter and combination with linear regression model. Non-linear regression model with Artificial Neural Network (ANN) modelling showed better fitting than linear regression model as significantly higher R^2 value (> 0.8) was obtained for each model developed. The R+G+B model ($R^2 = 0.9029$; RMSE = 1.1506 mg/L) was the best model to predict chlorophyll content in *Spirodela polyrhiza* whereas the B model was the best model for both *Lemna minor* ($R^2 = 0.9033$; RMSE =

0.3375 mg/L) and *Wolffia arrhiza* (R^2 = 0.9624; RMSE = 1.1180 mg/L). The mass of duckweed plantlets could be predicted well with linear regression model while the prediction of chlorophyll content in duckweed plantlets could be performed using developed ANN models with high accuracy.

CHAPTER 1

INTRODUCTION

1.1 Background

Image analysis is a fundamental tool used to recognize, differentiate, and quantify diverse types of images such as grayscale, color images, multispectral and hyperspectral images (Mendoza & Lu 2015). Pandurng and Lomte (2015) noted that the image analysis has been widely used in agriculture as an effective tool for non-destructive analysis of agricultural objects. The areas of agriculture where image analysis technique can be applied includes crop management, identification of nutrient deficiencies and plant content, fruit quality inspection, sorting and grading, crop and land estimation, as well as object tracking (Pandurng & Lomte 2015). According to Prabha and Kumar (2014), image analysis technique primarily used to analyse the quantitative and qualitative information collected from an image followed by statistical analysis which can be done through graphs and other statistical measurement tools. The procedures of image analysis include feature extraction, image segmentation, classification, measurements and interpretations (Prabha & Kumar 2014). Image analysis technique is becoming increasingly important these days due to its faster, more convenient, non-invasive as well as low-cost performance on products and processes (Prats-Montalbán et al. 2011).

The physical features of plants often provide important information on their growing environment and condition such as the content of pigments in plants can indicate the life processes of plants as plant pigments absorb various wavelengths of light which control the growth and rapid responses of the plants to the environment (Zielewicz et al. 2020; Bayat et al. 2018). While the evaluation on various physical features of plant is essential to examine the status of plants, the conventional method to conduct the measurement often involves the destruction of plant samples and complex procedures. For example, the procedures for the quantification of chlorophyll content in plants include extraction, centrifugation, and storage (Pocock et al. 2004). Hence, the utilization of image analysis could be an alternative for this.

In the recent years, there are several studies that utilizes image analysis technique on duckweed species. Duckweed, with the scientific name *Lemnaceae*, is the smallest flowering plant on Earth and it is a free-floating, aquatic green plant widely found in lentic or slowly moving water bodies (Ali et al. 2016; Asolekar et al. 2014). It is a useful organism that is often used for bioassay experiments involving water samples due to its fast growth rate and abundancy. The study carried out by Mazur et al. (2018) has implemented *Lemna minor* as biomonitoring tool to analyse the concentrations of toxins on surface waters and the negative effect of aquatic toxicants imposed on the growth and surface area of the duckweed. The result of the study showed that the growth of *Lemna minor* decreased at high concentration of toxicants as indicated by the decrease in surface area of the plant. Another study that applied image analysis technique on *Lemna minor* was carried out by Haffner et al. (2020). Image analysis technique was used to examine the negative effect of $K_2Cr_2O_7$ on *Lemna minor* bioassay with image analysis using two parameters, which were the number of leaves grown and the area occupied by the leaves. From the study, it was found that measuring the area of plantlets was faster and more precise compared to the leaves counting method.

Furthermore, Nesan and Chan (2019) applied image analysis technique to investigate whether the rate and degree of leaf color change of *Spirodela* *polyrhiza* plantlets which were exposed to copper was proportional to the concentration of copper present in the growth medium. The study found that the application of image analysis technique in this assessment required further improvement as there was limitation in the detectable range of values, which was between 1.25 mg/L and 5 mg/L of copper although empirical relationship between copper and leaf color was shown. Moreover, Tan, Ibrahim and Chan (2021) conducted a study to test the possibility of using smartphone to capture images for subsequent image analysis where the mass, chlorophylls, and anthocyanins content of *Spirodela polyrhiza* were predicted. The outcome of the study showed that the mass and color pigments of *Spirodela polyrhiza* can be predicted with sufficient accuracy and minor errors by using a smartphone camera.

Besides image analysis on macroscopic level, there were several studies that utilized image analysis technique on microscopic images of plants. Higaki (2017) had outlined the method of quantitative evaluation of the orientation, parallelness, bundling, and density of cytoskeleton using ImageJ image analysis software and pointed that microscopic image analysis currently is an important and essential research method in the field of cell biology. Toda et al. (2021) proposed a platform that allows real-time stomata detection on wheat-related species which consists of a deep neural network model-based stomata detector, an upright microscope connected to a USB camera and a graphics processing unit (GPU)-supported single-board computer. This method of real-time analysis allows judgment of the quality of image analysis results during observation and can prevent the acquisition of low-quality raw microscopic images acquired on-site which are inappropriate for analysis.

Pospiech et al. (2021) employed three types of microscopic techniques, namely bright field, dark field and phase contrast to classify the pollens in honey into the appropriate taxa by using various pollen descriptors. The study found that all the microscopic methods can be used to classify pollen in honey into the appropriate taxa and the best technique for classification was phase contrast microscopy, where the highest correct classification rate (CCR) value was achieved. Biswas and Barma (2020) demonstrated a new large-scale three-fold annotated microscopy image dataset in deep learning (DL) framework to measure different cell microstructures of raw potato tubers such as cell size and shape, intercellular space, cell wall thickness, starch and cell density distribution. It was showed that data could be acquired efficiently and the area of microscopy plant cell analysis for DL-framework could be enriched.

1.2 Problem Statement

The feasibility of utilizing image analysis technique on macroscopic level for the quantitative evaluation on various physical features of plants has been proved by several researchers throughout the recent years. Since microscopic imaging allows a more detailed observation of the plant structures compared to macroscopic imaging, image analysis on microscopic level may result in a more accurate outcome in the quantification of physical features of plant compared to that on macroscopic level. Therefore, the prediction of mass and chlorophyll content in duckweeds using microscopic imaging via image analysis technique is explored in this study in order to determine if the accuracy of the quantification results of macroscopic image analysis could be enhanced by microscopic image analysis.

In this study, microscopic images of three duckweeds species, namely *Spirodela polyrhiza*, *Lemna minor* and *Woffia arrhiza* under microscope are captured by smartphone and are analysed by using image analysis technique for the estimation of mass and chlorophyll content in plant samples. Consequently, correlation between area and mass of duckweeds is established through linear regression analysis whereas correlation between color parameter as well as color combination and chlorophyll content in duckweeds are established through linear regression analysis and artificial neural network (ANN) based non-linear regression modelling.

1.3 Objectives

The objectives of this research are as below:

- To establish the correlation between area of duckweeds detected with image analysis and the mass of duckweeds.
- To establish the correlation between color parameter or color combinations and chlorophyll content in duckweeds estimated by image analysis.
- To predict mass and chlorophyll content in *Spirodela polyrhiza*, *Lemna minor* and *Wolffia arrhiza* through microscopic image acquisition and subsequent image analysis.

CHAPTER 2

LITERATURE REVIEW

In the previous chapter, it has been discussed about the importance of image analysis technique in the field of agriculture and recent studies that employed macroscopic and microscopic image analysis in the quantification of various plant features. This chapter presents the previous discoveries and reviews available from credible scientific records and references that are related to this final year project topic. This chapter covers the overview of image processing and analysis, color space model, outcomes from various studies on prediction of chlorophyll content in different plants and acquisition of microscopic images using mobile phone camera in terms of advantages, challenges and techniques.

2.1 Image Processing and Analysis

Image processing or digital image processing is the process of converting an image signal into a digital signal and processing it with a computer (Luo et al. 2018). The process of image processing and analysis includes image enhancement, noise reduction, segmentation, restoration, encoding, compression, and extraction of features (Luo et al. 2018).

2.1.1 Application

Image processing and analysis is widely applied in various fields nowadays. Major fields that involve the application of image processing and analysis are listed in **Table 2.1**.

Some applications of image processing technology are image sharpening and restoration, medical field, remote sensing, microscopic imaging, video processing, transmission and encoding, machine or robot vision, color processing, pattern recognition (Bindhu & Dr. Thanammal 2020).

Table 2.1. Application of image processing and analysis (Luo et al. 2018).

Field	Application
Physics and Chemistry	Spectrum Analysis
Biology and Medicine	Cell analysis; CT; X-ray analysis
Environment Protection	Research of atmosphere
Agriculture	Estimation of plants
Irrigation works	Lake, river and dam
Weather	Cloud and weather report
Communication	Fax; TV; phone
Traffic	Robot; products
Economics	IC-card
Military	Missile guidance; training

In aviation field, digital image processing technology is used in aircraft remote sensing and satellite remote sensing technologies, mainly through reconnaissance aircraft to a certain area of the Earth. Analysis of image is done in real time, and multiple digital image processing techniques are used to process judgment reading. In aerial shooting, the digital code can be stored in the air after the required photos are processed by image analysis, then the satellite passing over the area with the receiving station can pass through the processing center. (Luo et al. 2018)

In medical field, digital image processing plays an important role. It is commonly applied in Gamma ray imaging, Imaging in the ultraviolet band, X Ray Imaging, PET scan, Medical CT and Imaging in the microwave band (Gonzalez & Woods 2002). Besides, commonly used imaging techniques in contemporary medical field includes Computer Tomography (CT), Magnetic Resonance Imaging (MRI) and X-ray inspection

(Prabaharan et al. 2020). In the diagnosis of various human diseases through common available medical tests, common method involves acquisition of biomedical images from living beings which are then being used for clinical diagnostics, disease treatment and continuous monitoring (Prabaharan et al. 2020). After enhancement followed by using suitable analysis techniques, the biomedical images can be processed with high efficiency and objectively evaluated (Padmappriya & Sumalatha 2020). The application of medical imaging is important to observe and study the function and behavior of internal organs without the need of surgery (Prabaharan et al. 2020). Although several sensors can be employed to study and monitor the health condition of the human such as blood pressure, body temperature, air respiratory, glucose level and skin perspiration, molecular analysis study implementing microscopic images can be used for recognition of the symptom of the diseases (Prabaharan et al. 2020). Moreover, image processing and analysis is also used in clinical diagnosis of melanoma; for instance, digital dermoscopy which is a widely used non-invasive tool is utilized to render an improved dermoscopic image through combination of optical magnification and special illumination techniques (Gonzalez & Woods 2002). In addition, the best performance of image processing technique is resulted by using the ANN method as the background is neglected and only the required portion of an image that is needed is displayed (Gonzalez & Woods 2002). Two of the most common classes of image processing algorithms, image restoration and image enhancement are used in the context of medical imaging to improve images for visualization or to facilitate further analysis for either clinical or scientific applications (Prince et al. 2020).

Image processing technology is also applied in communications engineering which mainly focuses on the design of sound words and the analysis of image data (Luo et al. 2018). According to Luo et al. (2018), code compression is the most important part of the image processing technique in this field where some of the current coding techniques are adaptive network coding, transform coding, and wavelet transform image compression coding.

Human authentication by face and fingerprint biometrics is another field that highly involves the application of image processing technology. Since people nowadays are required to be verified as a valid individual in order to be able to access various facilities including ATMs, buildings, airports, labs and files, biometric which is an identity-based method could provide necessary security for these applications (Gonzalez & Woods 2002). Human features such as finger prints, voiceprints, face characteristics, retina images, iris features and signature are being utilized by biometric systems (Gonzalez & Woods 2002). In the application of image processing, the detection of frontal human faces is being focused by various face detection algorithms (Padmappriya & Sumalatha 2020).

In the field of remote sensing, image processing and analysis is applied for detecting infrastructure damages caused by an earthquake. Image processing plays an important role in damage detection because without the assistance of image processing, it would usually consume a longer time even if serious damages are focused on and it is not possible to be examined with human eye in order to estimate damages when the area affected by the earthquake is too wide. An image of the affected area is captured from the above ground and then it is analysed to detect the various types of damage done by the earthquake. (Bindhu & Dr. Thanammal 2020)

Moreover, image processing and analysis is used in pattern recognition for identifying the objects in images which is then followed by training of the system using machine learning for the change in pattern. Character recognition or optical character recognition (OCR) which is mechanical or electronic translation of images of either handwritten or printed text into machine editable text is the most cost effective and speedy method available for many document input tasks. It is also a wide area for researchers in pattern recognition, artificial intelligence and machine vision. (Bindhu & Dr. Thanammal 2020)

The agriculture is also one field that is implementing image processing and analysis where a number of plant traits is monitored in order to achieve a higher yield (Bindhu & Dr. Thanammal 2020). Crops will inevitably produce some pests and illnesses during the sowing and harvesting processes, which will negatively impact the harvest and financial well-being of farmers (Luo et al. 2018). Image processing technology is used to identify and analyze pests and diseases, as well as to extract their features in order to achieve the effect of intelligent identification and eliminate pests plus illnesses for the benefit of agriculture (Luo et al. 2018).

2.1.2 Benefits

One benefit of the technology of image processing and analysis is good reproducibility. Image information is recorded and saved in binary format. The real information can be guaranteed as long as the original information is accurate since the copying of image during processing will not cause any influence on the original image. Digital image processing technology also has higher processing resolution compared to analogue technology. As information is recorded in the form of pixel lattices, the storage accuracy of an image highly depends on the number of quantization bits used in the conversion and the current digital image. (Luo et al. 2018)

Besides, digital image processing technology has wide range of applications where it can be derived from a variety of sources when given the basic principles, from microbes to space images, from human skeletons to lakes and mountains. In addition, image processing technology is flexible as digital images can be used for any operation including non-linearity and linearity while traditional analogue images are only capable of being processed linearly due to the limitations imposed by the optical principles used to generate them. (Luo et al. 2018)

2.2 Color Space Model

The color model can be defined as the digital representation of possibly contained colors or the way that we can recognize color, where human can visualize color through its attributes such as hue, and brightness. Color models is a system for measuring colors that can be perceived by human and a process of combining different values as a set of primary colors in which typical color models have three or four color components. (Ibraheem et al. 2012)

2.2.1 RGB Color Model

An RGB color space consists of all possible colors that can be made from three colourants for red, green and blue (Nishad & Chezian 2013). Red, green, and blue brightness values scaled such as between zero and one are the most common color specification (Castleman 1998). It is considered as the base color model for most image applications since the acquired image does not need any further transformation for displaying in the screen (Ibraheem et.al. 2012). All color spaces can be derived from the RGB information supplied by devices such as cameras and scanners (Nishad & Chezian 2013).

A cube could be used to present RGB color space by normalized RGB color values in the range [0,1] with gray values on the main diagonal of the black values (0,0,0) and on the opposite corner which is the white values (1,1,1) (Castleman 1998). The color of each pixel can be represented by the location of a point in the first quadrant of three-dimensional color space (RGB-space) (Ibraheem et.al. 2012). **Figure 2.1** represents the RGB color space, where one axis of a three-dimensional coordinate system is defined by each of the primary colors and every color plots to a position in this space (Castleman 1998). The RGB colour bar is shown in **Table 2.2**.

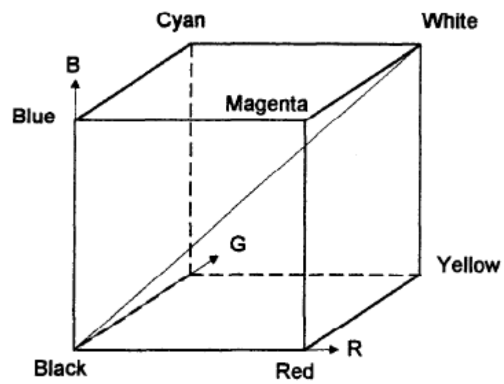

Figure 2.1. RGB color space (Castleman 1998).

Table 2.2. 100% RGB colour bar (Nishad & Chezian 2013).

	Normal Range	white	yellow	cyan	Green	magenta	Red	Blue	Black
R	0 to 255	255	255	0	0	255	255	0	0
G	0 to 255	255	255	255	255	0	0	0	0
B	0 to 255	255	0	255	0	255	0	255	0

From **Figure 2.1**, color black resulting from zero brightness of all the primary colors is represented by the point at the origin of RGB-space. On the other hand, color white appears from full brightness of all three primaries together, which is located at the comer diagonally opposite the origin of RGB-space. The primary colors, red, green, and blue constitute three of the corners of the color cube while the secondary colors, yellow, cyan (blue-green), and magenta (purple) constitute the remaining three comers. Besides, a shade of gray is produced by equal amounts of all three color components at lesser brightness. The locus of all such points falls along the diagonal of the color cube connecting the black and white points which is called “gray line”. (Castleman 1998)

Based on **Table 2.2**, each of the three primary additive colors, red, green and blue normally ranges from 0 to 255 that is R= {0, 1, 2 ….. 255}, G= {0, 1, 2 ….. 255} and B=

{0, 1, 2 ….. 255} whereas individual components are added together to form a desired color. Black color is produced when the amounts of three primaries which are Red, Green and Blue are in the minimum levels; while white color is produced when the amounts of three primaries are in the maximum levels. The basic rule of mixing in RGB color cube is as following: (Nishad & Chezian 2013)

$$R+G+B=White$$

$$R+G=Yellow$$

$$R+B=Magenta$$

$$G+B=Cyan$$

2.3 Prediction of Chlorophyll Content in Different Plants

There were various studies in recent years that pioneered image analysis technique as a non-destructive method for the prediction of chlorophyll content in plants. With different type of plant chosen to be used in each study, different methods were adopted in image acquisition and image processing with different outcomes and correlations between chlorophyll content and color parameters being established.

2.3.1 Correlation of Chlorophyll Content with Color Parameters

Many researchers had conducted studies on the correlation of chlorophyll content with color parameter in RGB color space obtained from color image analysis but different outcome was resulted for each study.

Studies by Yadav et al. (2010) and Gupta et al. (2012) on correlation between color parameter and chlorophyll content of micropropagated potato plants showed that that the color parameter B had positive correlation with chlorophyll content whereas R and G had negative correlation with chlorophyll content. Riccardi et al. (2014) who conducted the same correlation study on quinoa and amaranth leaves and Rigon et al. (2016) who researched on soybean obtained the same correlation for each of the color parameters.

In the research by Tan et al. (2021) on duckweed *Spirodela polyrhiza*, strong negative correlation with R^2 value above 0.95 was established between chlorophyll content of *Spirodela polyrhiza* with each color parameter R, G and B. The same correlation trends were obtained from chlorophyll content estimation study on rice by Hu et al. (2013).

In the correlation study conducted by Zhang et al. (2018) from on field maize, it was found that each of the R, G and B parameter had positive correlation with the chlorophyll content of field maize. Moreover, parameter R and G showed strong negative linear correlation with chlorophyll content of barley leaf with correlation coefficient value of 0.852 and 0.922 respectively whereas parameter B showed insignificant change with the increasing of chlorophyll content (Hu et al. 2020).

Based on study conducted by Damayanti et al. (2020) to predict chlorophyll content of cassava leaves, R parameter showed positive linearity pattern while G and B showed negative correlation linearity pattern.

From the results of studies from various researchers, it was found that the correlation of chlorophyll content with each of the RGB color space parameters varied with the type of plant.

2.3.2 Model with the Best Correlation with Chlorophyll Content in Different Plants

According to a study conducted by Barman and Choudhury (2020), in the application of linear regression to predict the chlorophyll of the leaves, there is only a few models composed of color indexes that showed a high correlation with the actual chlorophyll value of the citrus leaves. In linear regression, nine models of tender leaf showed an excellent correlation with the actual citrus chlorophyll, but only two of the models of immature leaves show a high correlation and one model showed a moderate correlation.

Another study conducted by Ali et al. (2012) on three different species which are lettuce, broccoli and tomato found that from the color image analysis, R+G appeared as having the best performance with the highest correlation coefficient among all RGB ratio tested for the correlation between RGB ratio and true chlorophyll content.

Based on study by Xu et al. (2021) on the prediction of chlorophyll content in *Astragalus* seeds, R and G are proved to be the ideal color index to be used for the prediction of chlorophyll content where linear relationship with high R^2 value was shown between each of these descriptors with the chlorophyll content of *Astragalus* seeds.

Moreover, Vesali et al. (2015) stated that previously reported studies found that color index, B was poorly fitted to chlorophyll content, while color index, R showed a good agreement with chlorophyll content. However, this study found that in all the images measured, G values were larger than R and B values, and the R index was almost twice of the B index.

Table 2.3 presents the comparison of model with the best correlation with chlorophyll content using linear regression model for different types of plant.

Table 2.3. Comparison of model with the best correlation with chlorophyll content using linear regression model for different types of plant.

Plant	Model	R^2	Reference
Tomato	Logsig = [G-R/3-G/3]/255	0.96	(Ali et al. 2012)
Lettuce	Logsig = [G-R/3-G/3]/255	0.89	(Ali et al. 2012)
Broccoli	Logsig = [G-R/3-G/3]/255	0.91	(Ali et al. 2012)
Pan Betel	NRI = R/(R + G + B)	0.95	(Dey et al. 2016)
Corn	14 Different Color feature	0.74	(Vesali et al. 2015)
Corn	VI	0.97	(Vesali et al. 2017)
Quinoa	RGB	0.97	(Riccardi et al. 2014)
Amarnath	RGB	0.96	(Riccardi et al. 2014)
Soybean	b color index of L*a*b	0.90	(Rigon et al. 2016)
Tender Citrus	NRI and VI	0.82	(Barman & Choudhury 2020)
Immature Citrus	R + G + B	0.75	(Barman & Choudhury 2020)
Mature Citrus	B and NBI	0.80	(Barman & Choudhury 2020)

Based on **Table 2.3**, it is clearly shown that the value of R^2 of the linear regression model varies with the type of plant leaves and the leaf color index. Therefore, only particular color parameters are correlated to the chlorophyll content in particular plant leaves.

2.3.3 Artificial Neural Network (ANN) Based Modelling for Prediction of Chlorophyll Content

There were several studies that employed MATLAB based linear modelling or Artificial Neural Network (ANN) based non-linear modelling to develop model for the prediction of chlorophyll content from color parameters due to low linearity in the linear regression plot of the correlation between chlorophyll content and color parameters.

Damayanti et al. (2020) used Artificial Neural Network (ANN) to produce model from RGB, HSV and HSL color space indexes for predicting chlorophyll content in cassava leaf. In their study, the 8-input model, 9 hidden layers with one output layer, in the proportion of 75% training data and 25% testing data was obtained as the best ANN model in predicting total chlorophyll, where the smallest MSE testing value of 0.092 and R testing of 0.8468 were obtained. Gradient Descent Backpropagation was used in training with the binary sigmoid function was used as activation function in the input and identity functions in the output layer. The highest source of total chlorophyll in cassava leaf was able to be maximally read with value of 84.68% by the model produced.

In the study by Gupta et al. (2012), the RGB color model parameters were not correlated with the actual chlorophyll content of micropropagated potato plantlets where the regression plot for each of the color parameter showed R^2 value of lower than 0.3. Linear modelling using MATLAB and non-linear modelling using ANN modelling were carried out in order to obtain models for to predicting chlorophyll content of leaves of micropropagated potato plantlets. For ANN based non-linear modelling, Easy-NN Plus v.7.0 was employed in creating neural network and a feedforward backpropagation neural network model was applied. Besides RGB color model parameters, mean brightness ratios (r, g and b) were also used in modelling which were calculated as r = R/R + G + B, g = G/R + G + B, and b = B/R + G + B. The models were developed with mean brightness (R, G, and B) or mean brightness ratio (r, g, and b) as input variables where either one was used at a time in a 3–1–1 network structure where 3 denotes the input variables, 1 represents the hidden neuron layer, and the other 1 represents the output. It was found that the linear and non-linear models had almost similar trend in the correlation between the predicted and actual chlorophyll content with R^2 value of 0.41 and 0.59 respectively for RGB model while R^2 value of 0.78 and 0.80 respectively for rgb model.

Mohan and Gupta (2018) used both linear and non-linear modelling to develop models to predict leaf chlorophyll content of rice from color parameters. A Feed Forward Neural Network with a backpropagation (BP) algorithm (FFNN-BP) was employed to develop a non-linear ANN model. RGB, rgb or dark green color index-mean brightness ratio (DGCI-rgb) was used as the input variables while chlorophyll content was used as the output variable in a 3–1–1 network structure. The ANN model was trained with 35 data and tested with 15 data obtained from rice leaves. The chosen of the optimum number of hidden neurons was on the basis of high R^2 and low RMSE values. The study found that the ANN model had better performance in terms of prediction of chlorophyll content than the linear model particularly with the RGB model where a poor correlation with R^2 of 0.335 was obtained for linear model whereas non-linear model had better correlation with R^2 of 0.797. For the rest of the color indices of rgb or DGCI-rgb, it was observed that both the linear and nonlinear model had equally well performance.

2.4 Acquisition of Microscopic Images Using Smartphone Camera

There are quite a number of studies recently that employed smartphone camera for microscopic image acquisition since this is a simple, economical, and highly practical technique to capture high quality images from a microscope.

2.4.1 Advantages

Normally, the capturing of image from a microscope needs a specially adapted microscope with a camera port, a specialized camera, an adaptor to attach the camera to the port and a computer system which are often either unavailable or insufficiently portable. The use of a mobile phone camera is more advantageous due to its affordability and availability, plus the handiness to carry at different sites, which makes it feasible to use as and when required. Most models of light microscope and smartphone camera can be applied in this image acquisition method. (Desai et al. 2014)

According to Barman and Choudhury (2020), the result of the captured images will not be changed with the switch of smartphone because the default condition of most of the smartphone cameras is similar. It was also mentioned that the images of the smartphone would not be affected by android version of the smartphone. Furthermore, the result of the method would not be affected by the original resolution of the leaf images as long as the size of the image is fixed where for this study is 300 x 400 (Versali et al. 2015). This is because the size of the image is independent of the actual size of the smartphone image (Versali et al. 2015).

2.4.2 Challenges

Despite the convenience and pros of using smartphone camera in capturing microscopic images, there are some challenges in using this method such as different ambient lighting conditions or shadows on leaves will affect the images (Versali et al. 2015). Yahya and Auba (2017) stated that the smartphone photomicrography requires a smartphone with a good rear camera that is at least 8 MP, a good binocular microscope, good ambient light, and steady hands. While the various phones provide similar morphological information, visible differences in resolution, brightness, and color balance still exist (Skandaraja et al. 2013). For example, the older mobile phones capture pixelated images with lower pixel count sensors and usually fail to capture detail of the target due to less well-corrected camera lenses incorporating fewer optical elements (Skandaraja et al. 2013). Besides that, in the study by Skandaraja et al. (2013), it was found that there was noticeably varying in the mean intensity and distribution of the cell colors and surrounding area across the images. Moreover, according to Yahya and Auba (2017), "vignetting" where the periphery of the circular image is slightly blurred is a minor drawback of smartphone photomicrography and zooming the image before capture or cropping out the blurred periphery can solve this problem.

2.4.3 Techniques

The capturing of images from microscope can be carried out by either a smartphone adapted to the eyepiece of microscope with new smartphone accessories to facilitate microscopic image capturing or a smartphone simply hold by free-hand (Morrison & Gardner 2014). There is a simple free-hand technique proposed by Morrison and Gardner (2014) to capture microscopic images which can be performed by using any smartphone camera. This method requires using of the third through fifth fingers of the left hand to steady the hand on the left microscope eyepiece, the thumb and second finger of the left hand and second through fifth fingers of the right hand to hold the camera between while leaving the right thumb free. The view beneath the microscope lens will eventually fill the screen or figure by slowly bringing the phone closer to the microscope, at the same time peering through the smartphone screen while focusing on the light in the ocular of the right eyepiece. The camera may be focused and the image can be captured by using the free right thumb. To remove vignetting which is the circular frame around the image, the camera's zoom function can be utilized.

CHAPTER 3

METHODOLOGY

3.1 Overview of Research Methodology

The workflow of the research on microscopic imaging prediction of mass and chlorophyll contents in duckweeds was presented in the form of a flow diagram as shown in **Figure 3.1**.

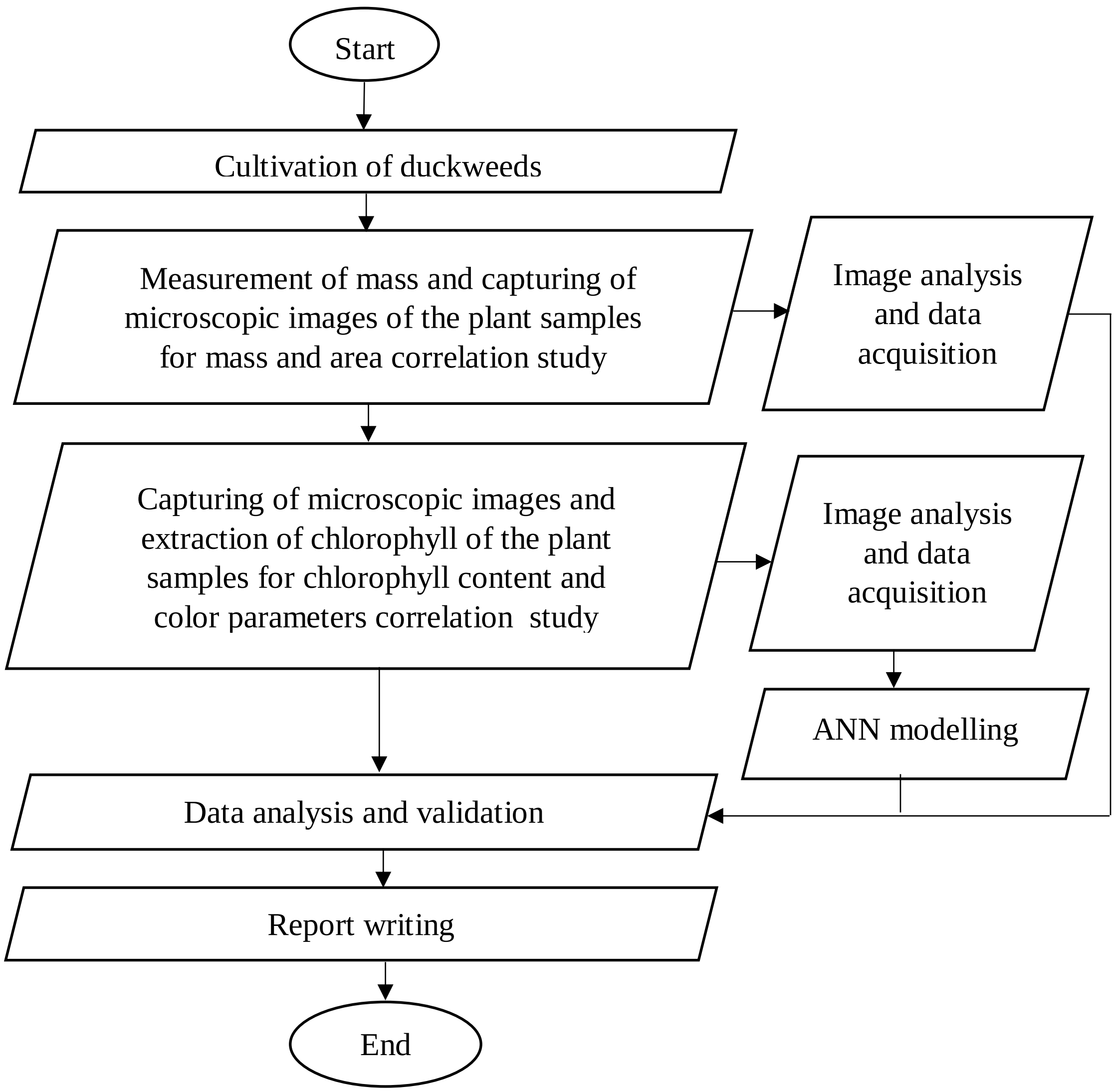

Figure 3.1. Flow diagram of research project on microscopic imaging prediction of mass.

3.2 Materials and Methods

The proposed materials and methods for the research on the estimation of mass and chlorophyll content of duckweeds using microscopic image analysis technique were adapted from Tan et al. (2021).

3.2.1 Establishment of Plant Samples

Cultivation of the three species of duckweeds, *Spirodela polyrhiza*, *Lemna minor* and *Wolffia arrhiza* was carried out with method modified from the aseptic culture established by Ng and Chan (2017). The duckweeds were maintained in Hoagland's medium containing 15 g/L sucrose under constant temperature of 25 °C, with 8000 lux light intensity and 24 hr photoperiod for 15 days (Ng & Chan 2017). The Hoagland's medium was prepared with components in the respective concentration as shown in **Table 3.1**.