Design and Test of Chlorophyll Fluorescence Image Acquisition System for Greenhouse Plant

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***Abstract* — To achieve the chlorophyll fluorescence image acquisition system for plants grown in greenhouse and accurately predict the chlorophyll fluorescence kinetic parameters, this paper built a prototype plant chlorophyll fluorescence image acquisition system by: i) utilizing computer machine vision technology to test the RGB color component, HSV indexes and the GRAY value of the marked leaves, ii) then modelling these image characteristics with chlorophyll fluorescence kinetics parameters via a) artificial neural network (ANN), b) support vector machine (SVM) and c) partial least squares regression (PLSR) methods. We analyzed and compared the prediction accuracy of the three models to fluorescence kinetic parameters, respectively, with different inputs of RGB with GRAY and HSV with GRAY. Our results showed that the three models could accurately predict the Y (Ⅱ), ETR, qL, NPQ and Fv/Fm parameters, and with input of RGB and GRAY, the prediction efficiency of the three models is generally superior to that with input of HSV and GRAY. It was the SVM model with input of RGB and GRAY that had the best prediction efficiency, with the correlation coefficient R of the Y (Ⅱ), ETR, qL, NPQ and Fv/Fm between predicted values and the real values were: 0.935, 0.941, 0.994, 0.987 and 0.941, the mean square deviation RMSE were: 0.013, 0.100, 0.036, 0.023 and 0.025. Our study indicated that the chlorophyll fluoresces image acquisition system could quickly and efficiently obtain and predict the plant leaf chlorophyll fluorescence image information and the chlorophyll fluorescence kinetic parameters as well, resulting in the monitoring and forecasting of plant health, plant environmental adaptation and plant photosynthetic performance.**

Keywords - chlorophyll fluoresces; image acquisition; prediction; modeling

I. INTRODUCTION

Chlorophyll fluorescence contains abundant photosynthesis information (mainly photosynthetic system Ⅱ, PS Ⅱ) that is more used in the study of the environmental stress of plant (light, temperature, nutrition, moisture, disease, etc.) as chlorophyll fluorescence could give a response to environmental stress in a quick and sensitive manner before plant phenotypes does so. It is named as the natural probe for plant physiological information NDT [1-4]. Today, the chlorophyll fluorescence detection devices that have been widely used are developed based on the theory of active induction of chlorophyll fluorescence. Most of them are made in German (Walz), U.S.A. (OPTI), British (Hansatech), Czech (FluroCam), etc. These instruments achieve measurement of chlorophyll fluorescence of a single leaf and an exploration into the characteristics of plant local growth. However, it takes long time for the instruments to measure the parameter in the test and measurement. On this account, study of a method with which plant chlorophyll fluorescence parameter can be predicted and obtained on a quick and effective basis is of vital significance in industrial promotion of chlorophyll fluorescence detection technique. With the development of computer technology, machine vision technology has been widely used in agricultural engineering circle [5-6]. However, existing study of machine vision in protected agriculture is applied to the building of a crop growth model. For instance, nutritional status [17-19] of crops are determined by measurement of stem width of plant [7-9], leap shape and size [10-12], plant height [13-14], plant population growth and development [15-16] or by obtaining color, spectral and morphologic features of crops. Application of machine vision technology to acquisition of chlorophyll fluorescence information of plant leaves starts late [20] and is less reported.

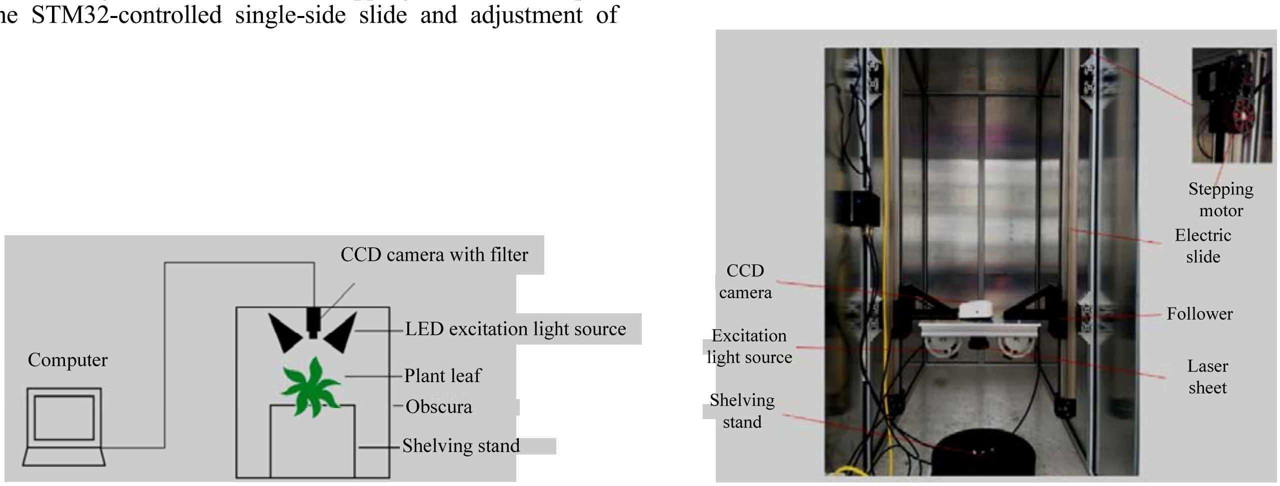
In this study, emission of fluorescence of plant leaves in the obscura is achieved by excitation of LED blue violet with the wavelength of 473nm according to the generation principle of chlorophyll fluorescence. The chlorophyll fluorescence image very sensitive to plant photosynthesis performance and growth environment is captured by the CCD industrial camera with filter. By extraction of RGB component, HSV indicator, and GRAY component of the fluorescence image and based on the MINI-PAMⅡ measured chlorophyll fluorescence parameter, the system achieves the function of predicting the chlorophyll fluorescence parameter of the plant by modeling. The study will be of important guiding significance in online diagnosis of effectiveness of plant photosynthesis, plant health and adaptability of growth environment in protected agriculture cultivation.

II. MATERIAL AND METHOD

A. *Composition of Fluorescence Image Acquisition System*

The plant leaf chlorophyll fluorescence image acquisition system consists of the upper-computer industrial computer, lower-computer STM32, obscura and LED blue violet excitation light source with the wavelength of 473nm, filter

(4600480nm), CCD camera as well as related controller and slides. The industrial computer in the system is the master control core of the system, with the communication enabled through serial interface and STM32 controller; height regulation of light source and camera and regulation of light source excitation intensity are achieved with positive/negative rotation of the stepping motor at the top of the STM32-controlled single-side slide and adjustment of luminance of the light source. The industrial computer uptakes fluorescence image by using LabVIEW software and virtual instrument controlled CCD camera. The image is processed and predicted online by calling Matlab through Matlab Script node. The test bed of chlorophyll fluorescence image acquisition system is shown in Fig.1.



a. Schematic diagram of platform b. Obscura internal physical diagram

Figure 1. Schematic & Physical diagram of chlorophyll fluorescence image acquisition system.

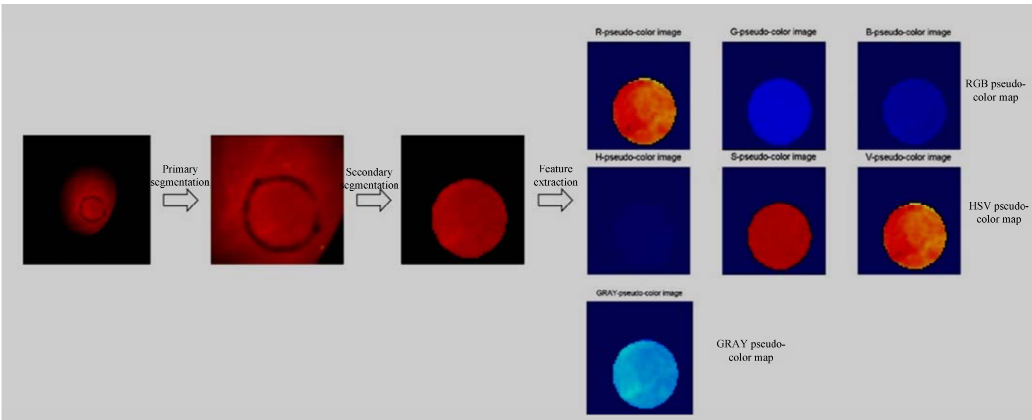


Figure 2. Image segmentation and extraction of image feature pseudo-color map

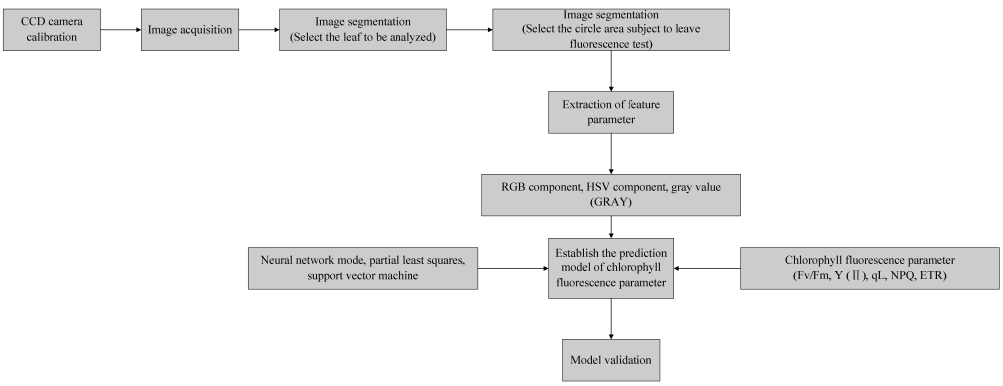


Figure 3. Establishment of prediction model of chlorophyll fluorescence kinetic parameter

B. Image Data Acquisition

In this study, the diameter of the circular ROI (region of interest) selected is 35 pixels. To fully compare the effects of different modeling methods on the predicted performance, the support vector machine (SVM), partial least squares regress (PLSR) and artificial neural network (ANN) are used for modeling analysis of data. The accuracy of prediction for the chlorophyll fluorescence kinetic parameter by the image acquisition system are determined by a comparison with the actual measured results. 70 groups of model training set samples and 30 groups of prediction set are used in the test. The extraction and prediction modeling processes of image information throughout the test are shown in Fig.2 &3.

C. Test Material

The test was carried out in Venlo in the College of Engineering of Nanjing Agricultural University from April 5, 2016 to May 5, 2016. The test material was Su pepper 5# (planted on March 29, 2016, purchased from Jiangsu Academy of Agricultural Sciences) under growth and planted in the greenhouse. It was under soil-less cultivation. The substrate selected was Galuku coconut dust. The nutrient solution used was Japanese horticulture nutrient solution formula (Table 1). 40 seedlings with roughly the same growth were randomly selected as the object in the test. Such seedlings were put into four groups each of which consists of 10 seedlings in order to obtain diversified chlorophyll fluorescence image information and kinetic parameter information. After one-week seedling recovering (April 5), nutrient solution with varying concentrations (water; 1.5mS/cm; 2.0mS/cm; 2.5mS/cm) was given to pepper seedlings. There was no any other environment stress in the course of pepper growth. After test treatment for one month, parameter measurement was started.

TABLE 1. ELEMENTS COMPOSITION OF JAPANESE HORTICULTURE NUTRIENT IN 1L DEIONIZED WATER

Element composition	Dosage (g L-1)
$Ca(NO_3)_2 \cdot 4H_2O$	850
KNO_3	910
$NH_4H_2PO_4$	155
$MgSO_4 \cdot 7H_2O$	500
Na_2Fe-EDTA	25
H_3BO_3	3
$MnSO_4 \cdot 4H_2O$	2
$ZnSO_4 \cdot 7H_2O$	0.22
$CuSO_4 \cdot 5H_2O$	0.05
$(NH_4)_6MO_7O_2 \cdot 4H_2O$	0.02

Upon measurement, 25 fully unfolding canopy leaves without mechanical damage were randomly selected from different treatment groups, and a circular mark with the diameter of 1cm was provided at the leaf center near to the vein for measurement of fluorescence induction parameter and acquisition of fluorescence image components.

For measurement of chlorophyll fluorescence parameter, MINI-PAMⅡ produced by German Walz is used in acquisition. Before acquisition, plants are put under dark adaptation for 30 min, followed by acquisition of the fluorescence parameters of the marked locations of each leaf. The acquisition parameter settings of the fluorescence instrument are: the measured light intensity is 4, the frequency is 3, the saturation light intensity is 10 and the step size is 13. Representative chlorophyll fluorescence parameters of the leaf are: Fv/Fm (maximum photochemical quantum yield, to reflect the proportion of quantum involved in PSⅡ photochemical reaction to the absorbed light quantum of PSⅡ), ETR (relative electron transfer rate, to reflect the effective photosynthesis rate), Y(Ⅱ) (actual photochemical quantum yield of PSⅡ, to reflect the part of light-excited energy involved in PSⅡ photochemical reaction), qL (photochemical quenching coefficient, to reflect the openness of PSⅡ reaction center) and NPQ (non-photochemical quenching coefficient, to reflect the proportion of the absorbed light quantum that is consumed in the form of heat) are calculated automatically by the instrument.

III. MODELING AND ANALYSIS

A. Establishment of ANN model

The generalized regression neural network (GRNN) is a neutral network model with a 4-layer network structure with obvious advantages in the learning rate and approximation capability. In this paper, GRNN is used as the tool, 4 quantitative chlorophyll fluorescence image variables are used as the network inputs: R component, G component, B component ad GRAY value; H component, S component, V component and GRAY component, and 5 chlorophyll fluorescence parameters in corresponding leaf area are used as the network outputs: Y(Ⅱ), ETR, qL, NPQ and Fv/Fm to establish the ANN model that expresses the quantitative relations between chlorophyll fluorescence parameters and image components. Network training is given at the sample modeling point value. The neural transfer functions of the selected model layer, summation layer and output layer are Formula (1), (2), (3) & (4), respectively. The initial default value of SPREAD is 1:

$$P_i = \exp\left[\frac{(X - X_i)^T (X - X_i)}{2\sigma^2}\right] i = 1, 2, \ldots, n \quad (1)$$

$$S_D = \sum_{i=1}^{n} P_i \; i = 1, 2, \ldots, n \quad (2)$$

$$S_{Nj} = \sum_{i=1}^{n} y_{ij} P_i \; j = 1, 2, \ldots, k \quad (3)$$

$$y_j = \frac{S_{Nj}}{S_D} \quad j = 1, 2, \ldots, k \tag{4}$$

Note: *n*-number of input neuron, number of sample learning; *k*-dimensionality of output neurons; X -network input variable; X_i -learning sample of neuron *i*; P_i -output of neuron *i* on the model layer; S_D -arithmetic sum of all neuron outputs on the model layer; S_{Nj} -weighted sum of all neuron outputs on the model layer; y_{ij} -connection weight of neuron *i* on the model layer and summation neuron *j* on the summation layer; y_j -output prediction result of neuron *j* on the output layer

B. *Establishment of SVM Model*

SVM prediction modeling is done with LIBVM 3.1 tool. Common modeling methods may not yield a relatively accurate regression diagnostic model. The radial basic RBF kernel function, i.e. Formula (5) is used. SVM function of RBF kernel function is: the boundary function c and kernel bandwidth g are obtained by cross validation.

$$K\,(x, x_i) = \exp\left(-\gamma\left(x - x_i\right)^2\right) \tag{5}$$

C. *Establishment of PSL Model*

The basic practice of partial least squares regression is that: 1. the first component t_1 (t_1 is the linear combination of x_1, …, x_m) is extracted from the independent variable set and the first component u_1 is extracted from the dependent variable set (y_1, …, y_p) and that the maximum correlation between t_1 and u_1 should be achieved; 2. The regression of dependent variable y_1, …, y_p and t_1 is established. If the regression equation achieves satisfactory accuracy, the algorithm will come to an end. If not, extraction of the second pair of components should continue till satisfactory accuracy is achieved.

D. *Validation of Model Reliability*

As shown in Table 2, in the prediction models based on RGB and GRAY, SVM model has the best prediction of chlorophyll fluorescence parameters. In the test set, the correlation coefficient R between predicted and measured values of Y(Ⅱ), ETR, qL, NPQ and Fv/Fm and mean square error RMSE are 0.0943/0.013, 0.941/0.100, 0.994/0.036, 0.987/0.023 and 0.991/0.025, followed by the effect of prediction by PLSR. GRNN prediction effect comes in the last place: in the test set, the correlation coefficient R between predicted and measured values of Y(Ⅱ), ETR, qL, NPQ and Fv/Fm and mean square error RMSE are 0.881/0.105, 0.853/0.512, 0.989/0.059, 0.986/0.044 and 0.977/0.047. In the prediction models based on HVS and GRAY, SVM achieves the best prediction of chlorophyll fluorescence parameters, followed by PLSR and GRNN prediction effect comes in the last place.

Table 3 indicates that in two input modes, SVM model has a smaller relative error RE. The minimum RE value occurs when RGB and GRAY are used as the inputs. The model has certain prediction capability for Y(Ⅱ) and ETR. It can accurately predict qL, NPQ and Fv/Fm. To sum up, establishment of SVM prediction model based on RGB and GRAY can predict chlorophyll fluorescence parameters of plant leaves both quickly and accurately.

IV. CONCLUSIONS

1) In this study, a simple plant leaf chlorophyll fluorescence image acquisition system is established in the generation principle of chlorophyll fluorescence. The system can quickly capture fluorescence image information of the plant online. With the use of GRNN, SVM and PLSR, a prediction model is established by using the fluorescence image RGB/GRAY and HSV/GRAY as the inputs and chlorophyll fluorescence parameters (Y(Ⅱ), ETR, qL, NPQ, Fv/Fm) as the outputs so that the system has the function of predicting chlorophyll fluorescence parameters of plant leaves online.

TABLE 2. PREDICTION ACCURACY OF DIFFERENT PREDICTION MODELS ON CHLOROPHYLL FLUORESCENCE PARAMETERS

Model		Y(Ⅱ)		ETR		qL		NPQ		Fv/Fm	
		R	RMSE	R	RMSE	R	RMSE	R	RMSE	R	RMSE
RGB+GRAY	GRNN	0.881	0.105	0.853	0.512	0.989	0.059	0.986	0.044	0.977	0.047
	SVM	0.935	0.013	0.941	0.100	0.994	0.036	0.987	0.023	0.991	0.025
	PLSR	0.884	0.015	0.769	0.465	0.991	0.046	0.959	0.031	0.981	0.037
HSV+GRAY	GRNN	0.830	0.057	0.729	1.261	0.889	0.059	0.958	0.042	0.944	0.050
	SVM	0.883	0.035	0.837	0.055	0.959	0.034	0.964	0.032	0.977	0.017
	PLSR	0.832	0.046	0.743	0.480	0.917	0.046	0.955	0.033	0.967	0.020

Note: R denotes the coefficient of correlation. RMSE denotes the standard error.

TABLE 3. PREDICTION ABILITY OF DIFFERENT PREDICTION MODELS ON CHLOROPHYLL FLUORESCENCE PARAMETERS

Model		Y(Ⅱ)		ETR		qL		NPQ		Fv/Fm	
		RE/%	RPD	RE/%	RPD	RE/%	RPD	RE/%	RPD	RE/%	RPD
RGB+GRAY	GRNN	2.65	1.62	1.84	1.88	5.19	6.48	2.35	3.17	1.91	1.67
	SVM	1.28	1.80	1.14	1.57	5.05	6.45	1.33	3.84	1.22	2.43
	PLSR	1.43	1.60	1.77	1.54	3.31	9.23	3.13	3.46	1.22	1.99
HSV+GRAY	GRNN	2.45	1.45	5.02	1.43	5.45	6.58	3.42	2.69	2.05	1.55
	SVM	0.92	1.66	0.86	1.50	2.52	1.76	3.40	4.31	1.53	1.86
	PLSR	1.50	1.49	1.93	1.47	3.50	9.23	3.40	3.26	1.53	3.66

Note: RE denotes the relative error; PRD denotes the performance index of model. For PRD≤1.4, it indicates that the model is incapable to predict the mock object. For 1.4＜PRD≤2.0, it indicates that the model requires further improvement. For PRD≥2.0, it indicates that the model performance may accurately predict the object.

2) The results of prediction model analysis show that the SVM model established by using RGB and GRAY as the inputs achieves the best prediction error effect. It provides an accurate prediction of Y(Ⅱ), ETR, qL, NPQ and Fv/Fm. The correlation coefficient R between predicted and measured values is: 0.935, 0.941, 0.994, 0.987 and 0.991; the mean square error RMSE is: 0.013, 0.100, 0.036, 0.023 and 0.025, which demonstrates that the fluorescence parameter information can be effectively predicted with this chlorophyll fluorescence image acquisition system. Further, it can monitor and anticipate plant health, adaptation to the environment and photosynthesis performance.

3) What the chlorophyll fluorescence image captures is the initial fluorescence intensity image of dark-adaptation leaves subject to excitation by excitation light. Fluorescence parameters are predicted with an analysis of the image information of ROI particular region. On this account, the overall feature is reflected with local fluorescence image feature of the leaf, which may result in prediction errors to an extent. In addition, the prediction models established with different algorithms largely rely on the front-end training data, thus leading to a change of the model with the changing training set. For this reason, pre-training must be provided in different scenarios of application in order to minimize the prediction error.

REFERENCES

[1] Li X, Lu W, Hu GY, et al. Effects of light-emitting diode supplementary lighting on the winter growth of greenhouse plants in the Yangtze River Delta of China[J]. Botanical Studies, 2016, 75:2.

[2] Janka E, Körner O, Rosenqvist E, et al. High temperature stress monitoring and detection using chlorophyll a fluorescence and infrared thermography in chrysanthemum (Dendranthema grandiflora)[J]. Plant Physiol. Biochem., 2013, 67:87–94.

[3] Long JR, Ma GH, Wan YZ, et al. Effects of nitrogen fertilizer level on chlorophyll fluorescence characteristics in flag leaf of super hybrid rice at late growth stage[J]. Rice Sci., 2013, 20:220–228.

[4] Woolery PO, Schmal JL and Davis AS. Evaluation of chlorophyll fluorescence as an indicator of dehydration stress in American chestnut seedlings[J]. Native Plants J., 2010, 11:27–32.

[5] Chen YR, Chao KL, Kim M． Machine vision technology for agricultural applications[J]． Computers and Electronics in Agriculture, 2002, 36:173－191.

[6] Ishizuka T, Tanabata T, Takano M, et al. Kinetic measuring method of rice growth in tillering stage using automatic digital imaging system[J]. Environment Control in Biology, 2005, 43(2): 83－96.

[7] Meyer GE, Davison DA. An electronic image plant growth measurement system[J]. Transactions of the ASAE, 1987, 30(1): 242－248.

[8] Yunseop K, David MG, Johnny P, et al. Hyperspectral image analysis for water stress detection of apple trees[J]. Computer and Electronics in Agriculture, 2011, 77(2): 155－1607.

[9] David S, Murat K, Chieri K, et al． Lettuce calcium deficiency detection with machine vision computed plant features in controlled environments[J]. Computers and Electronics in Agriculture, 2010, 74(2): 238－243.

[10] Liu JG, Elizabeth P, Stuart A． Assessment of in situ crop LAI measurement using unidirectional view digital photography[J]. Agricultural and Forest Meteorology, 2013, 169: 25－34.

[11] Calvoálvarez E, Stamatakis K, Punzón C, et al. Infrared fluorescent imaging as a potent tool for in vitro, ex vivo and in vivo models of visceral leishmaniasis.[J]. Plos Neglected Tropical Diseases, 2015, 9(3).

[12] Lu S, Lu X, Zhao W, et al. Comparing vegetation indices for remote chlorophyll measurement of white poplar and Chinese elm leaves with different adaxial and abaxial surfaces.[J]. Journal of Experimental Botany, 2015, 66(18): 5625-5637.

[13] Devmalkar V S, Murumkar C V, Salunkhe S M, et al. Studies on pigment chlorophyll isolation and estimation of different bryophytes for their biochemical properties[J]. Journal of Natural Product & Plant Resources, 2014.

[14] Naoya Takahashi, Keiya Hirota, Yoshitaka Saga. Facile transformation of the five-membered exocyclic E-ring in 132-demethoxycarbonyl chlorophyll derivatives by molecular oxygen with titanium oxide in the dark[J]. Journal of Porphyrins & Phthalocyanines, 2015, 19(4): 1-7.

[15] Zhang Y, Wagle P, Guanter L, et al. Phenology and gross primary production of maize croplands from chlorophyll light absorption, solar-induced chlorophyll fluorescence and CO2 flux tower approaches[J]. J.mater.chem.a, 2015, 3(18).