# Simulating the fluorescence under natural conditions by Fluspect model and comparing simulated fluorescence spectra to FluoWat measurements

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February,2016

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# Simulating the fluorescence under natural conditions by Fluspect model and comparing simulated fluorescence spectra to FluoWat measurements

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Enschede, The Netherlands, February,2016

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# ABSTRACT

Chlorophyll fluorescence (ChlF) has become a useful indicator to detect plant photosynthesis and stress conditions. Many techniques have been used to measure fluorescence. Fluorescence spectra can also be simulated with the Fluspect model. The Fluspect model is a radiative transfer model that simulates the leaf reflectance, transmittance and upward and downward fluorescence. Fluspect has hitherto mostly been compared to data were measured under artificial light. The Fluspect model has not been validated using field measurements where fluorescence is excited by natural sunlight. In this study, healthy maize and soybean plants grown under natural conditions were used in the experiments. The FluoWat devices can obtain spectral signatures from leaf, which can measure sun-induced reflectance (R), transmittance (T) and chlorophyll fluorescence (ChlF) on both the illuminated and shaded side of the leaf. Simulated reflectance and transmittance by Fluspect model were compared to the measured data by the FluoWat system. The RTM inversion model is utilized to retrieve the leaf parameters from measured reflectance and transmittance data. Using Fluspect model one can simulate the chlorophyll fluorescence by vegetation parameters. Both retrieved parameters and simulated fluorescence were compared to measurements, in order to validate the model for natural conditions. The simulation fluorescence show a good correlation with the measured data in all wavelengths ($R^2$>0.9). However, there is a lager error at the peak of the fluorescence curve. Evaluating by other index, the relative error between simulated and measured fluorescence is higher than 150% around the first peak. To improve the model, the inversion of the emission efficiency parameters for photosystem II and I (FQE II and I) code has been added to the model. This code retrieves the FQE II and I by using the measured fluorescence spectra The Fluspect model with the FQE inversion code has reduced the error at the peak of the curve. The parameters without the photosystem II and I are almost same with the parameters which have been retrieved by the mode without the FQE inversion code. That means the photosystem II and I play a dominant role in producing the fluorescence. However, the photosystem II and I are the characteristics of the plants, but also depend on the natural condition. The measurement of the emission efficiency of the photosystems for the photosystem II and I is complex. The value of the photosystem II and I cannot be easily measured in this study. So the model with new code is still needed to test and verify with photosystem II and I measurement.

***Keywords:*** *Sun-induced fluorescence; model inversion; Fluorescence simulation; FluoWAT*

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# 1. INTRODUCTION

## 1.1. Background

Photosynthesis is one of the most important biological processes on Earth. Sun is a common energy source for photosynthesis. The vegetation absorbs photosynthetically active radiation (PAR) and uses for photosynthesis. The absorbed PAR is not fully used by photochemical process. Part of it is released primarily by heat radiation and a small part of radiation as fluorescence (see Figure 1- 1 The Photosynthesis Process). The fluorescence is one of the most useful indicators of vegetation status. The fluorescence is sensitive to physiological parameters. The example is that the terrestrial vegetation's carbon budget and GPP require information on physiological parameters, which can be obtained from fluorescence emission. So many studies pay more attention to fluorescence simulation to get the vegetation status (Verhoef et al., 2000).

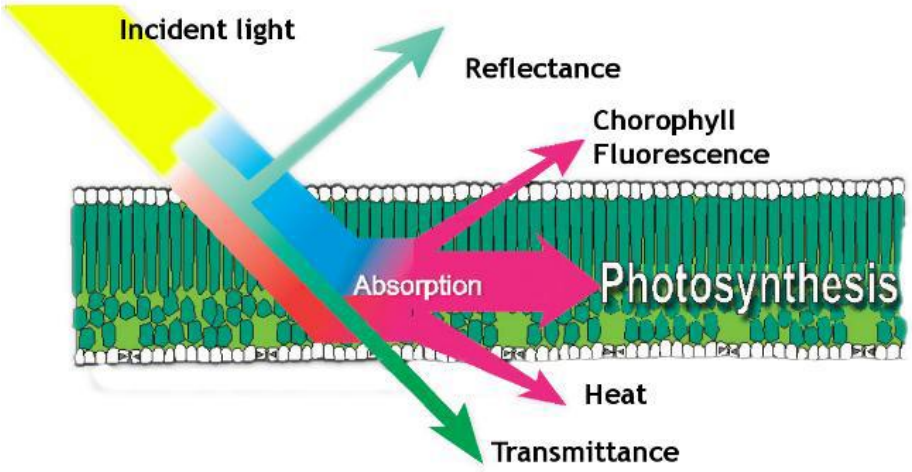



Figure 1- 1 The Photosynthesis Process
Source: http://ipl.uv.es/flex-parcs/

The fluorescence is the emission of light by a substance that has absorbed light or other electromagnetic radiation. Normally, the fluorescence is much weaker than the reflected solar radiation (about 2-5% in the near infrared), both fluorescence and reflectance are included in the red/far-res region (650-800 nm) of the vegetative reflectance spectrum (Lichtenthaler et al., 1986). Vegetation fluorescence is emitted from the foliage through the photosystem. In the visible region of the spectrum : The peaks of fluorescence occur at 686, and 740 nm (Lichtenthaler et al., 1986).

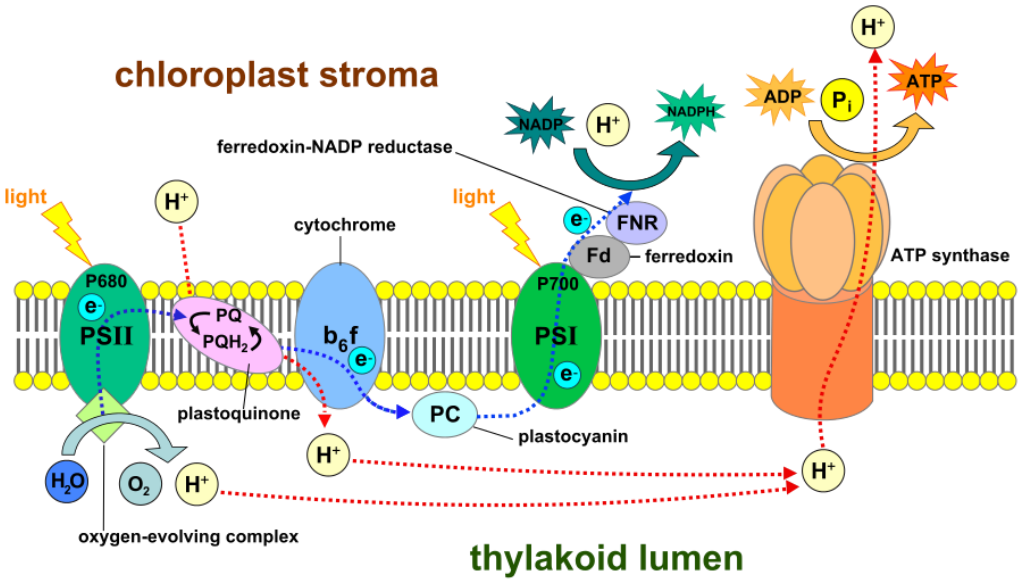



Figure 1- 2 Photosystem II and I
Source: http://www.mhhe.com/

Fluorescence will continuously be produced by the photosystems while the plants absorb the incident light. Louis Duysens first proposed the concepts of photosystems I and II in 1960. But the significance of these discoveries was not yet known (Fromme et al., 2004). The absorption of light and the transfer of energy and electrons are termed the light reaction. Photosystem II is responsible for the emission of the fluorescence around the 680nm, while fluorescence of photosystem I peaks around 700nm. The contributions of the two photosystems show as peaks in the fluorescence spectra. The emission efficiencies of photosystem I and II (FQE value in the model) will influence the simulated fluorescence. The peaks in the fluorescence simulation curve will change by the changing the values of the FQE II and I. There are two main categories of techniques to measure ChlF: laser induced fluorescence technique (LIT) and solar induced fluorescence (SIF). The LIT technology uses a laser beam to excite the specie (a molecule or atom) in the plant. After absorbing the laser beam photons, the specie will be in a short-lived excited electronic state. In order to return to a stable condition, the molecule releases the extra energy in several pathways: fluorescence, heat or used for photochemistry (Sun et al., 2003). In this technology, fluorescence can directly reflect information about the molecular structure. The fluorescence of a leaf is not only affected by vegetation fluorescence spectral properties, but also pigment composition, pigment content and plant biomass. The LIT is a good indicator of plant photosynthesis. This technology has the limitation that it cannot be used at large scale and vegetation monitoring by LIF from satellite platform not possible yet (Rosema et al., 1997). For the solar induced fluorescence technology (SIF), passive detection of fluorescence is used to estimate the vegetation properties. The detector will measure the signal which is produced by plants themselves. The RS data is measured by the UAV and satellite. With the reflectance and the fluorescence data from the remote sensing, the retrieval techniques are meant to separate the two. However, the RS data is too coarse spatial resolution for the simulation model. The input data are needed more detailed and require field-scale data(Hall et al., 1992). There is also indirect relationship between radiative transfer parameters and model parameters as SVAT model because of different scholarly background. So it is not possible to directly translate (van der Tol et al., 2009).
To provide available data and verify Fluspect model simulating the fluorescence, the portable device FlouWat has been used to detect the fluorescence more accurately. The FlouWat is utilized to measure reflectance (R), transmittance (T) and Fluorescence (F) by hyperspectral spectroradiometer in upward and

downward positions in turn (Van Wittenberghe et al., 2015). The FlouWat is applied to measure the leaf R-T-F under natural condition. Than the Fluspect model has been presented to simulate the fluorescence with more accurate and easier data.

### 1.2. Research problem

The research problem is that Fluspect model has been used in canopy model such as SCOPE and validated with the artificial light, but the Fluspect has not been validate under natural condition. So the research is aimed to test and verify if the Fluspect model can simulate the fluorescence in natural conditions. The simulation R, T and ChlF data are still need to be compared with the measured data. The measured data are verified the efficiency for simulating the fluorescence.

### 1.3. Research questions

1. Do simulated and measured fluorescence have a good correlation?
2. Is the relative error between the simulation result and the measured value below 50%?
3. In evaluating the model, do the peak values around 687nm and 740nm in simulated fluorescence curves match to the measured data?
4. Can the Fluspect inversion model be improved by adding the FQE inversion code to get more accurate simulations of fluorescence?

# 2. MATERIAL

### 2.1. Study area

The study area is in Campus Klein Altendorf, located about 100 km from Aachen in Germany. There is a greenhouse measurement facility with maize and soybean inside. Maize and soybean (*zea mays and Glycine max*) were also grown outside. Two varieties with different leaf chlorophyll concentrations (high and low) were measured. The high-chlorophyll canopy was the wild type, and the low-chlorophyll the MinnyGold variety. Both maize and soybean were unstressed and well developed. Measurements were taken at the top, middle and the bottom of the plant. Measurements at the bottom of the canopy were difficult to obtain due to lack of solar light. The leaf was pulled out in sunshine to do the measurement. But in the natural condition, the leaves on the bottom always grow in dark condition. When give the sunlight for the leaf on the bottom, the leaf will suffer from excess light. The measurement for these leaves perform not well. In this study, we chose the leaves on the top and the bottom which are normally exposed in the sunlight.

a. The study area b. The plants in study area

Figure 2- 1 The study area

## 2.2. Field experiment

### 2.2.1. FluoWat

For the instruments, the FluoWat with a spectrometer have been used to measure the reflectance, transmittance and fluorescence. The filter in instrument can cut off the incident light between the 650nm to 880nm.

Figure 2- 2 The FluoWat Device (Wittenberghe et a., 2014)

The FluoWat is a portable device to measure upward and downward leaf emission under natural conditions. After light adaptation, the measurement can be done, but not on a constant moment of the day. The FluoWat has one spectroradiometer which will be used in two positions, one upward and one downward of the leaf (Fig.3A). In both positions, the fiber optic points to the leaf surface vertically. An open aperture is designed to let solar beam enter at relative 45° position. So the reflectance and transmittance can be obtained with upward and downward fiber optic (Fig.3B). Then, a short-pass filter restricts the light entering the open aperture and cut off the light above 650nm. The upward and

downward fluorescence can be measured by two probes, respectively (Fig.3B). By this way, the reflectance, transmittance and fluorescence can be measured.

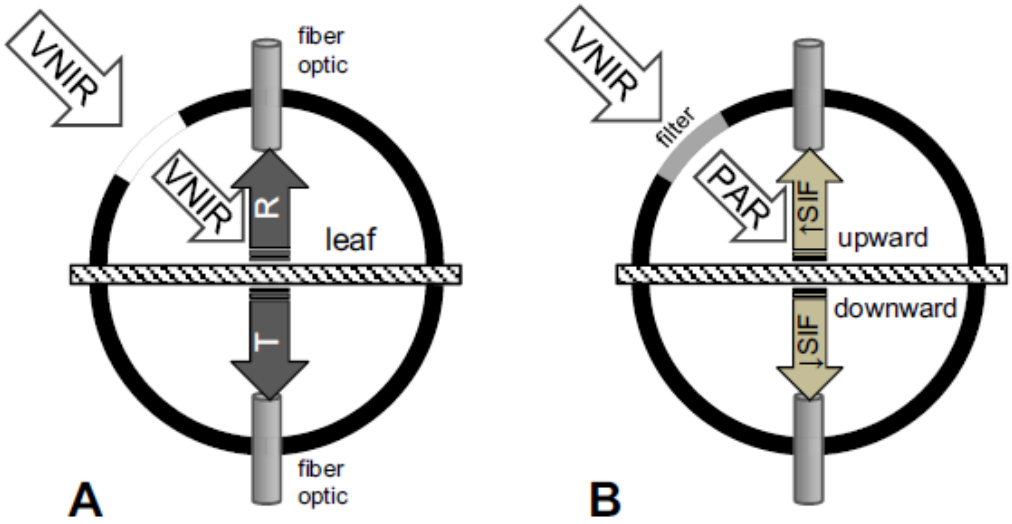


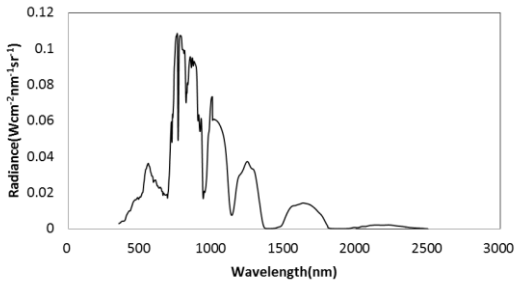

Figure 2- 3 Scheme of FluoWat leaf clip(Wittenberghe et al., 2014)

### 2.2.2. Measurement protocol

Measurements were taken by research staff of the Forschungszentrum Jülich and PhD students of the University of Twente on two varieties of soybean plants (Glycine max): a high and a low chlorophyll variety. Measurements were taken on 3 plants per variety, 3 canopy levels per plant, 1-2 leaves per level one or two and always only 1 leaf for level 3. That makes 18 - 30 leaves, depending on the day and measurement. On some days, only the top layer was measured.

## 2.3. Collection data

Eight measurements have been done for every leaf. Every measurement will measure a kind of radiance.
1. Unfiltered radiance (Figure 2- 4) is radiance which is reflected by the reference board. The reference board can reflect the total light without absorption. The unfiltered radiance is the total incident light.
2. Filtered (cut of at ca 650nm) radiance (Figure 2- 5) is the light which is cut off the incident light from 650nm to 800nm.




3. Leaf UP (
Figure 2- 6) means the radiance from the leaf at upward direction. It contains the reflectance and the emission of the leaf.
4. Leaf UP with filter (Figure 2- 7) is the radiance from the leaf at upward direction. From the 650nm to 800nm, it only has the emission of leaf. The incident light in filter wavelength has been cut off.
5. Leaf DOWN with filter (Figure 2- 8) means the radiance in the downward direction. The transmittance from the incident has been cut off by the filter from the 650nm to 800nm.

6. Leaf DOWN (Figure 2- 9) means the radiance which has been measured in the downward direction. The radiance contains the transmittance of the light and the emission of the leaf.
7. Unfiltered radiance (Figure 2- 10) is the same as the first figure. It is used to make sure that the sunlight does not change during the measurement.
8. Filtered radiance (Figure 2- 11) is the same meaning with the previous figure.

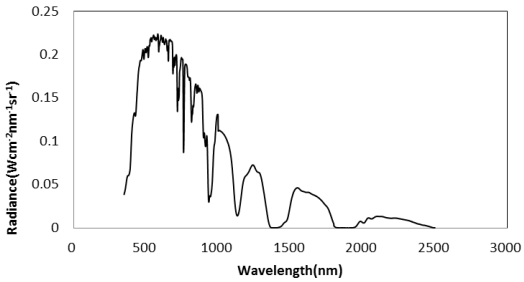



Figure 2- 4 Unfiltered Radiance

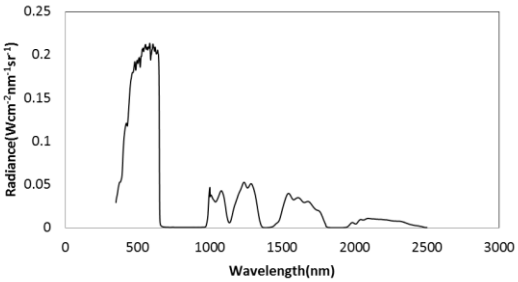



Figure 2- 5 Filtered Radiance

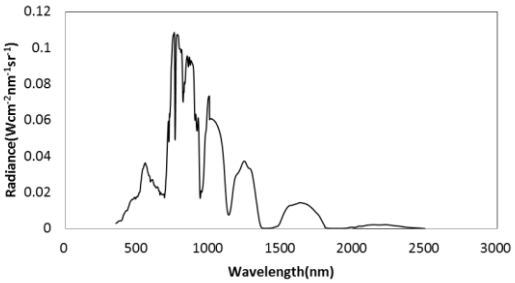



Figure 2- 6 Leaf UP

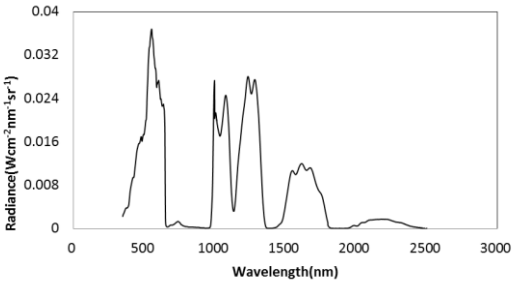



Figure 2- 7 Leaf UP with filter

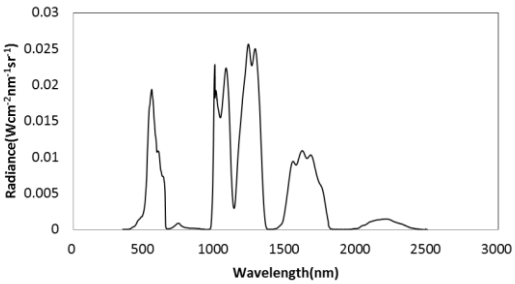



Figure 2- 8 Leaf DOWN with filter

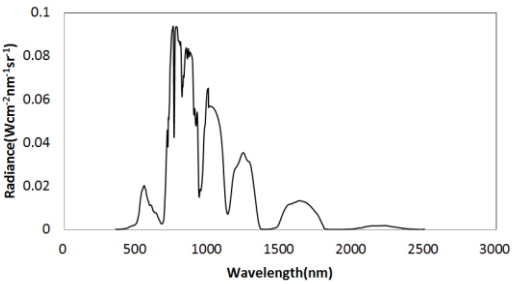



Figure 2- 9 Leaf DOWN

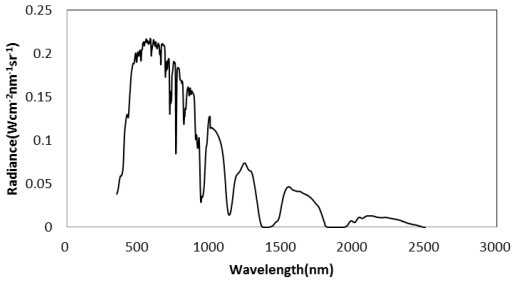



Figure 2- 10 Unfiltered radiance

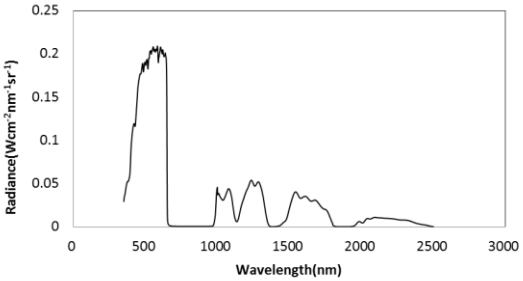



Figure 2- 11 Filtered Radiance

To ensure high quality data, the measurements were repeated 5 times for each leaf. So each measurement consists 5 files, each file is an average of 5 measurements, so for one leaf that is 40 files total in the sequence described above.
The reason for five times measurements in one leaf is to keep the accuracy of measurements. Solar radiance can change within seconds, so if you measure at the beginning and in the end, you make sure the measurement was ok. If the solar light changes like a cloud blocks the solar beam during the measurement, the result will change. The whole set of recording 40 files for one leaf can last up to a few minutes. The weather can change in that time.
In other words, radiance should not change from beginning to end to ensure data quality. After checking if the input files are correct, it can be verified whether the data are accurate, than the simulation can be done.

## 2.4. Model description

A radiative Transfer Model (RTM model) is used to describe the path of radiation in the medium (air, water, urban area, vegetation). The radiative transfer model is based on the fundamental equation of radiative transfer. The RTM model can simulated leaf reflectance and transmittance using leaf properties data. The model also can simulate fluorescence from vegetation properties and leaf structure (Nastassia Vilfan et al., 2016).
Fluspect is a radiative transfer model which can simulate the reflectance, transmittance and fluorescence. This model is one part of SCOPE model. SCOPE model (Soil Canopy Observation, Photochemistry and Energy fluxes) is a vertical integrated radiative transfer and energy balance model (van der Tol et al., 2009). The theory of radiative transfer, micrometeorology and plant physiology is main principle of the SCOPE model. The model is good at interactions of different model components. Three different models are contained in the main model:
1. The model FLUSPECT is used for optical properties of leaves and combines the properties with a photosynthesis model;
2. The model also calculates heterogeneous canopy and soil temperatures with the energy balance model;
3. The model also considers the irradiance, canopy temperature and other environmental conditions to calculate chlorophyll fluorescence, which was only calculated by a function of irradiance in previous model.
These part of model can use independent or together. The model is modular and several components of model can be replaced by other model.
As one part of SCOPE model, the Fluspect model is based on the PROSPECT model and contains an additional module to calculate the Fluorescence matrix for both side of leaf. This model has been used for the global fluorescence data simulation.
The RTM inversion model is a means of inversion of the Fluspect mode. RTM inversion model retrieve leaf composition and structure from measured reflectance and transmittance data. The RTM inversion model is built for the purpose of estimating the terrestrial surface properties from remote sensing data since the reflectance data can be measured by satellites over the Earth. A limited numbers of input parameters are needed for both sub models with a little computation time. By inverting the Fluspect model, we can estimate the leaf and canopy parameters.
The RTM inversion model uses a direct inversion parameters. Perhaps the simplest way of inverting a model is using look-up-table. A LUT is built in advance. A search operation is built to find the suitable parameters combinations between the measured and LUT spectra. However, a sufficiently large LUT are needed to achieve high accuracy (Combal et al., 2002; Tang et al., 2007; Weiss et al., 2000). So the direct inversion is more convenient to do the simulation. The inversion contains equations to invert the results. Without a large LUT, it is fast to calculate the results.

For solving the inversion model, we need to define an objective function ξ, for example: $(r_{mod} - r_{meas})2$. With a priori parameter value, $r_{mod}$ and ξ are calculated. Then we adjust parameters and calculate $r_{mod}$ and ξ again. If the ξ is smaller, the new ξ can be accepted. Otherwise, we adjust parameters differently and go to the last step. In the end, we obtain the parameter value that result in the best matching spectrum (show in Figure 2- 12).

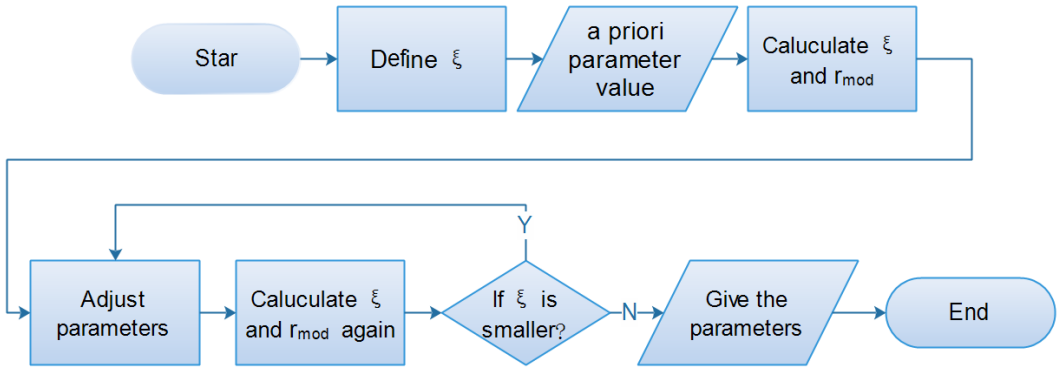



Figure 2- 12 The inversion flow chart

# 3. METHODOLOGY

In order to simulate the fluorescence in natural conditions, a new method has been presented to do simulation with measured reflectance and transmittance data and compare with the measured data to evaluate this method (see Figure 3- 1). This process contains two different steps to simulate the chlorophyll fluorescence. The method is first done by retrieving leaf composition and structure from the measured reflectance and transmittance by the RTM inversion model applied to Fluspect. Then Fluspect model can simulate the upward and downward fluorescence with vegetation parameters and plant structure. The simulation results were compared with measured fluorescence.

With the precision data obtained with the FluoWat, this method is reasonable to simulate the ChlF with the reflectance and transmittance data under natural conditions. This method has been test and verified in the artificial conditions. For the natural condition, the method is still needed to be tested. The method efficiency was tested and verified in different aspects like the correlation between simulation and measured fluorescence curves and the peak values.

For improving the model, the emission efficiency of Photosystem II and I can be taken into consideration. The photosystem plays an important role in the emission of fluorescence. When the plants do the photosynthesis, photosystem II is responsible for the emission of the fluorescence around the 680nm, while fluorescence of photosystem I peaks around 700nm.The last version of Fluspect model uses doubling algorithm that generates the fluorescence matrices of the leaf to do the simulation. The reflectance and transmittance data were applied to do the simulation along with the fluorescence spectra of Photosystem II and I as a basis for the doubling algorithm. For the improvement of model, the FQE II and I retrieving code has been added into the model to simulate the fluorescence more accurate.

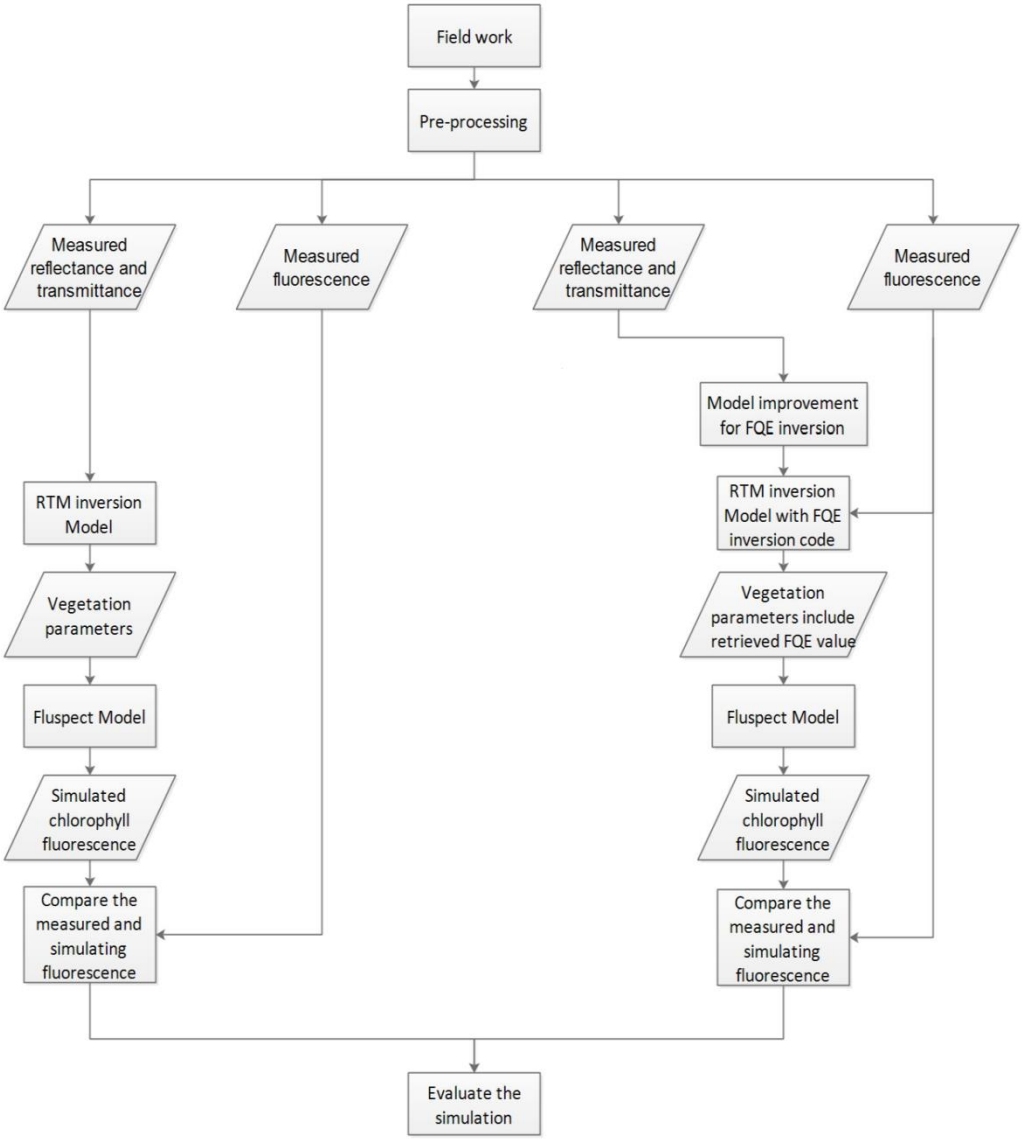



Figure 3- 1 The methodology flow chart

### 3.1. Pre-processing

After getting the eight different measurements in one sequence, the reflectance, transmittance and the fluorescence were calculated. The pre-processing is the process to deal with the initial data and calculate the data which we need. In this processing, we will calculate the $\tau$, $R_a$, $T_a$, $F_u$ and $F_d$.

$$\tau = \frac{I_f}{I} \tag{1}$$

$\tau$ is the transmittance of the filter. The value means the rate of light which can go through the filter. It also is on the behalf of the ability of the filter cut off the light.

$$R_a = \frac{E_u}{I} \tag{2}$$

$R_a$ is the apparent reflectance. This is proportion between the light irradiates to the face of the light and reflect to the top direction. Through the equation, the reflectance can be calculated.

$$T_a = \frac{E_d}{I} \tag{3}$$

$T_a$ is the apparent transmittance. It is the rate how much light can go through the leaves to the bottom side.

$$F_u = \frac{E_{uf} - E_u\tau}{1-\tau} \quad (4)$$

$$F_d = \frac{E_{df} - E_d\tau}{1-\tau} \quad (5)$$

*Fu* and *Fd* are the fluorescence at the illuminated side of the leaf [$Wm^{-2}\mu m^{-1}sr^{-1}$]. Equation 4 and 5 shows how they are calculated from the radiance coming from the leaf. The equation will wipe out the light which is not cut off by the filter. The results can be shown in the Figure 3- 2 The emission of fluorescence up and Figure 3- 3 The emission of fluorescence down.

For the further processing, the true reflectance (R) and transmittance (T).were calculated as:

$$R = R_a \quad for \;\; wl < 650\,nm \; or \; wl > 850nm \quad (6)$$

$$R = R_a - F_u \quad for \;\; 650nm < wl < 850nm \quad (7)$$

$$T = T_a \quad for \;\; wl < 650\,nm \; or \; wl > 850nm \quad (8)$$

$$T = T_a - F_d \quad for \;\; 650nm < wl < 850nm \quad (9)$$

After the pre-processing, the measured fluorescence can be obtained. With the reflectance and transmittance data, the model can simulate the fluorescence, which will be compared with the measured fluorescence.

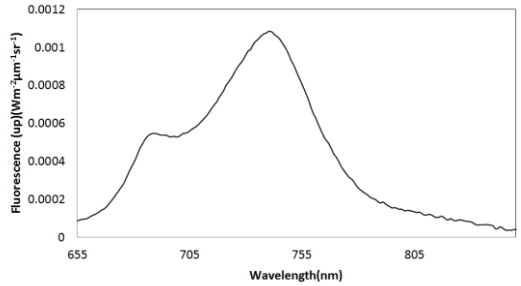



Figure 3- 2 The emission of fluorescence up for one leaf after per-processing

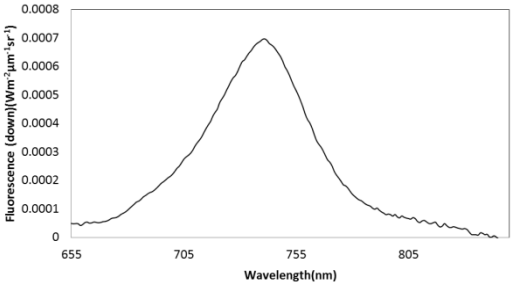



Figure 3- 3 The emission of fluorescence down for one leaf after per-processing

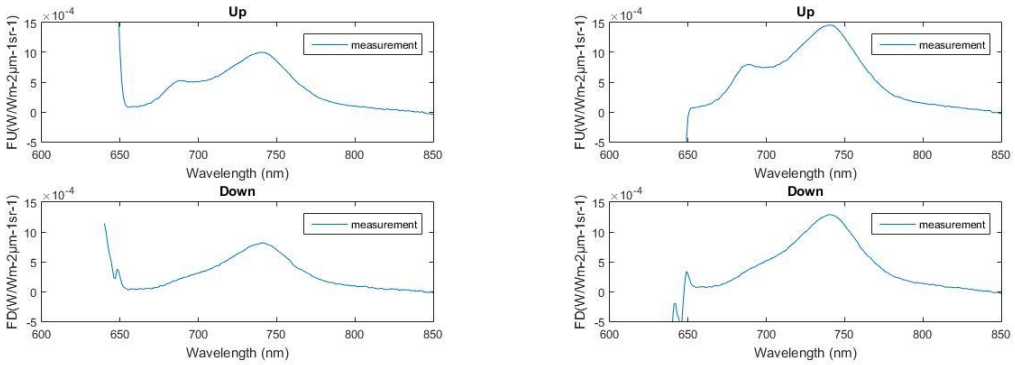



Figure 3- 4 The fluorescence error in measurement

After the per-processing, some fluorescence values appear to be negative (shown in Figure 3- 4). However, negative fluorescence is physically impossible. In the natural condition, the fluorescence is either zero or greater than zero. The negative values are caused by the fact that at the beginning spectral range of the filter, the filter does not work very well. Normally, the filter will cut off the light from 650nm. But it is hard to achieve this aim. The $\tau$ cannot decrease to 0 immediately from the 650nm (see Figure 3- 5). There are still some point between 1 and 0. When calculate the fluorescence, the value of $E_u\tau$ may be larger than the $E_u$. so the value $E_u$- $E_u\tau$ may be negative. Than upward and downward fluorescence were calculated as a negative value after doing the pre-processing.

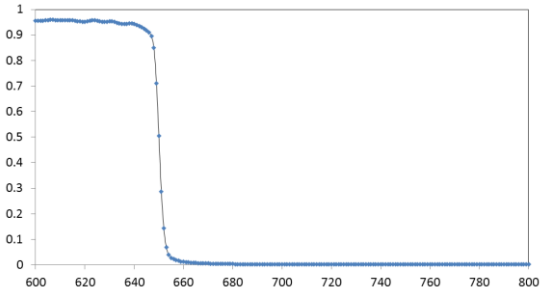


Figure 3- 5 The per-processing parameter τ

Some fluorescence values at the 650nm are very large. Because the filter will cut off the light from the 650nm to the 800nm, the 1-$\tau$ will be closer to 0 before the 660nm. The $Fu$ and $F_d$ were calculated by dividing 1-$\tau$. So the upward and downward fluorescence will be a large value before the 650nm.
These errors will be solved by removing fluorescence values before 660nm. Fluorescence was replaced by zero before 660nm. There have a very small influence on the model evaluation due to the most emission fluorescence from 660nm to 800nm.

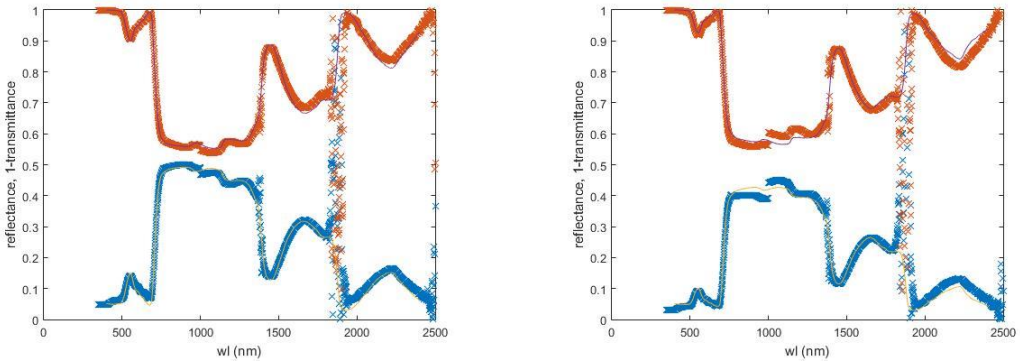



Figure 3- 6 The interval in reflectance and transmittance control figures

For the reflectance and transmittance data, there are three intervals because of the detectors. The data in there intervals will have a little measurements errors (shown in Figure 3- 6). For some measured data, the reflectance and 1- reflectance curves overlap. The overlap of the detectors' wavelength will lead to the first two jumps. Because fluorescence will appear from 680 to 800 wavelength region, the intervals will not influence the results a lot.

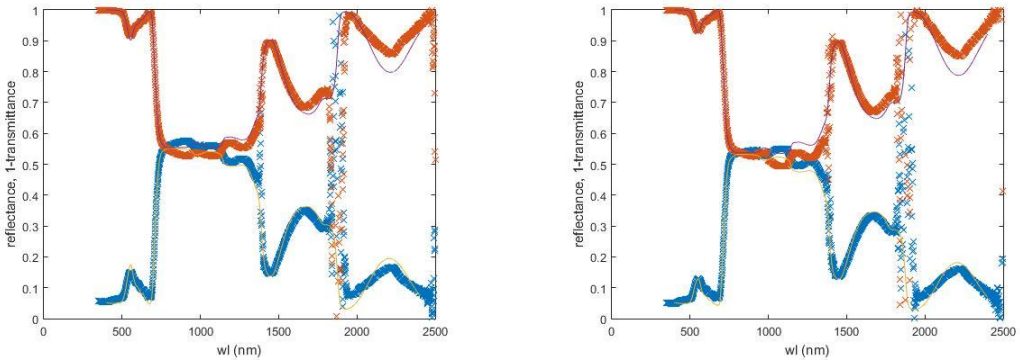



Figure 3- 7 The overlap in reflectance and 1-tranmisstance table

In some measurements, the percentages of measured reflectance and 1-transmittance curve overlap from 800 to 1400nm (shown in Figure 3- 7). That is due to the fact the leaves have non-lambertian reflectance. The light will be reflected as diffused reflectance. The detector in the top will received part of the reflected light. As some part of reflectance was missed, the absorption will be less. In extreme cases, the absorption will smaller than 0 and the curves overlap. The data with this obvious measurement error will be excluded from the simulation.

### 3.2. Model generation

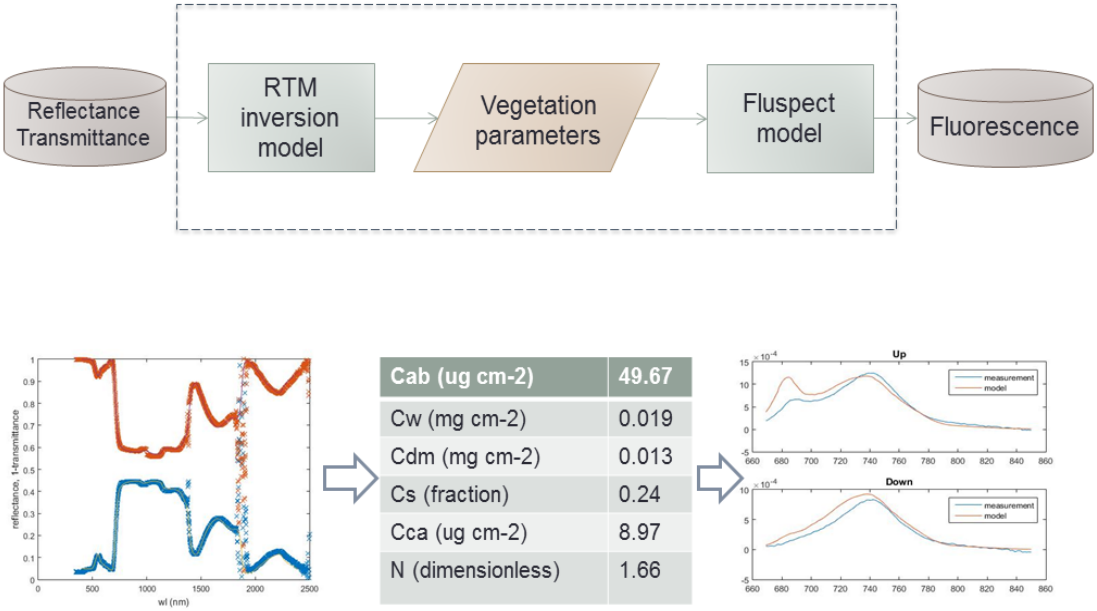



Figure 3- 8 The sequence of steps in the process of fluorescence simulation

With RTM inversion and Fluspect models we can simulate the fluorescence at the backward (the illuminated side) and forward (shaded side) direction. The model also can put the results on one figure to compare to the measured data. The RTM inversion model can simulate the vegetation parameters first. The parameters contain 6 different parameters to be retrieved (shown in the Table 3- 1). The photosystem II and I emission efficiencies will be replaced by FQE II and I. These two parameters do not affect the reflectance and de transmittance. The FQE was maintained at default values in the simulation, not automatically adjustable before the model improving.

With the reflectance and the transmittance data, the model can simulate the vegetation condition and shown as the vegetation parameters. It is easy to identify the high chlorophyll content leaves and the low chlorophyll content leaves.

The Fluspect model can simulate the fluorescence with these parameters with the vegetation parameters. With the 6 different vegetation parameters, the fluorescence can be simulated. Every parameter has the influence on the simulation. The FQE parameters which set as fixed values will influence two peaks of fluorescence emission in the ChlF curve. With these 8 parameters, the model can simulate the fluorescence. Table 3- 1 shows the vegetation parameters which have been inverted and used for simulating the fluorescence.

This method contains the Fluspect inversion and Fluspect model which work together to find the best inversion results and compare simulation results with the measured data.

Table 3- 1 The vegetation parameters in model retrieving

| | | |
|---|---|---|
| Cab | chlorophyll content | [μg cm$^{-2}$] |
| Cdm | dry matter content | [g cm$^{-2}$] |
| Cw | leaf water thickness equivalent | [cm] |
| Cs | senescent material | [fraction] |
| Cca | carotenoids | [μg cm$^{-2}$] |
| N | leaf structure parameter | [] |
| FQEII | Fluorescence quantum yield efficiency of Photosystem II. | |
| FQEI | Fluorescence quantum yield efficiency of Photosystem I. | |

### 3.3. Improvement of model

For the model improving, there is a key point to improve the model. Model works well with the R2 and PEARSON index which show that the simulation and measured data have high correlation. However, comparing the peak values of the fluorescence, the difference is higher than expected (over 100%). The peaks are on behalf of the photosystem II and I (FQE II and I).

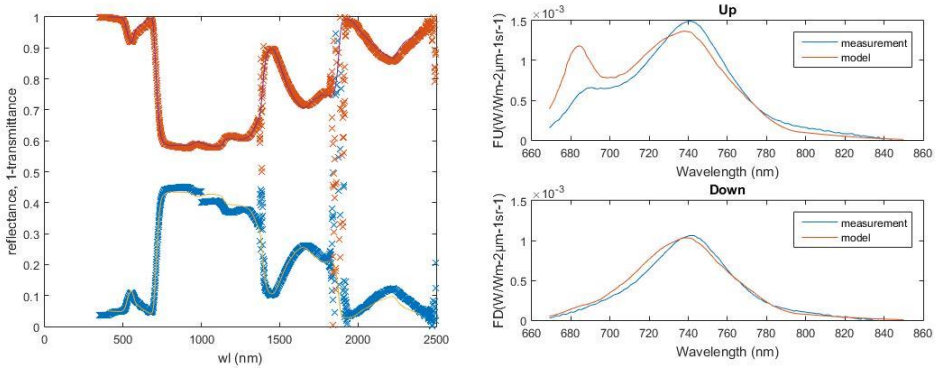
Normally, we use the empirical coefficients to do the simulation, because it is hard to measure these two parameters. The big problem in measurement is that the signal of photosystem II and I are mixed. It is hard to identify each by simple measurements. And they may vary with weather conditions such as illumination and temperature. The default FQE II and I are not accurate in simulation.

The main solution is that add the FQE inversion code into the model to tune the coefficients to the measured fluorescence. With the inverting of the FQE parameters, the model can work more accurate and find the best curve to fit the measured value. It helps to explain model deficiencies.

With adding the code, the model can invert the FQE value. Photosystem II and I are the functional and structural units of protein complex in photosynthesis producing the fluorescence. It is worth to pay attention to the FQE value. It will have an important influence on the fluorescence up and down. The FQE1 will control the fluorescence on the second peak in fluorescence. The FQE2 will influence the first peak on the fluorescence. With the inversion of these two parameters, the results will show the simulating value of FQE1 and 2. The simulation fluorescence will be more accurate. Also, the fitted values of FQE1 and FQE2 can give information about the physiology of the plants.

### 3.4. Model validation and comparison

The model simulation results are evaluated by the PEARSON and $R^2$ indexes. These two parameters are used to measure the correlation between two variables. The Figure 3- 9 shows the comparison results. These two parameters can give a standard to evaluate the model.




PEARSON=0.94
R^2=0.89

PEARSON=0.98
R^2=0.97

Figure 3- 9 The Comparison Results

### 3.4.1. Coefficient of determination

With the $R^2$ the simulation fluorescence can be compared with the measured fluorescence, the correlation can be analysed between the simulation data and the measured data. After the correlation analysis, the model evaluation can be done.

There is a fluorescence value from 650 to 800nm in every wavelength. Every wavelength will give a number as *i*. The n is accumulated by the number of *i*. A simulation fluorescence data set has n values marked y1...yn (collectively known as *yi*), each associated with a measured fluorescence *f1...fn* (known as *fi*, or sometimes *ŷi*).

If $\bar{y}$ is the mean of the observed data:

$$\bar{y} = \frac{1}{n}\sum_{i=1}^{n} y_i \tag{10}$$

Then the variability of the data set can be measured using three sums of squares formulas:

The total sum of squares (proportional to the variance of the data):

$$SS_{tot} = \sum_i (y_i - \bar{y})^2 \tag{11}$$

The regression sum of squares, also called the explained sum of squares:

$$SS_{reg} = \sum_i (f_i - \bar{y})^2 \tag{12}$$

The sum of squares of residuals, also called the residual sum of squares:

$$SS_{res} = \sum_i (y_i - f_i)^2 \tag{13}$$

The most general definition of the coefficient of determination is

$$R^2 \equiv 1 - \frac{SS_{res}}{SS_{tot}} \tag{14}$$

In statistics, the coefficient of determination is an index that indicates how well data fit the model. It provides a measure of how well measured data are fitted by the model, as the proportion of total variation of outcomes explained by the model. The simulation fluorescence in each wavelength will be compared with the measured data from 640 to 800nm. If $R^2$ is 1, the number indicates that the simulation data fit the data very well. If the $R^2$ is 0, that means the simulation data does not fit the data at all. The condition can be because the simulation data has no relationship with the measured data. Normally, the value of $R^2$ is neither 1 nor 0. If the $R^2$ is larger than 0.9, we can still say that the simulation fluorescence fits the measured date well in fluorescence range. The model works well in simulation.

### 3.4.2. Pearson correlation coefficient

In the field of natural science, the Pearson correlation coefficient is widely used for measuring the correlation degree between two variables. The comparison between the simulation results and the measured data can be done by Pearson correlation coefficient.

Unlike the Coefficient of determination (which is scaled from 0 to 1), this coefficient measures how highly correlated are two variables and is measured from -1 to +1. Similar to the modified Coefficient of determination, a Pearson Correlation Coefficient of 1 indicates that the simulation data objects are perfectly correlated with the measured data, while a score of -1 means that the simulation data objects are negatively correlated with the measured data. In other words, the Pearson Correlation score quantifies how well two data objects fit. If the simulation results have negative correlation with the measured data, it can describe by the value.

There are several benefits to using this type of Pearson correlation coefficient. The most important one is that the accuracy of the score increases when data is not normalized. The fluorescence data is not normalized. As a result, this metric can be used in the fluorescence data.

In essence, the Pearson Correlation score finds the ratio between the covariance and the standard deviation of both objects. In the mathematical form, the score can be described as:

$$Pearson(x,y) = \frac{\sum xy - \frac{\sum x \sum y}{N}}{\sqrt{(\sum x^2 - \frac{(\sum x)^2}{N})(\sum y^2 - \frac{(\sum y)^2}{N})}} \quad (15)$$

### 3.4.3. Absolute and relative error

Absolute error is the difference between the simulation value $x_0$ and its actual value or measured value $x$, given by:

$$\Delta x = x_0 - x \quad (16)$$

It is specified in both the size of the error and also indicates its positive and negative directions. Absolute error can reflect the size of the deviation that simulation results from the measured value in the same unit dimensional, which represents the exact size of the actual deviation from the true value. In this study, the absolute can represent the actual deviation between the simulation fluorescence and the measured fluorescence.

The relative error is the ratio that the absolute error accounted for the equivalent of measured value, given by

$$\delta x = \frac{\Delta x}{x} * 100\% \quad (17)$$

It will give a percentage and it is a dimensionless value. In general, it is better to reflect the simulation credibility by the relative error.

In the model simulation, the simulation fluorescence can minus the measured data to calculate the absolute error. To better explain the results, the relative error can be calculated.

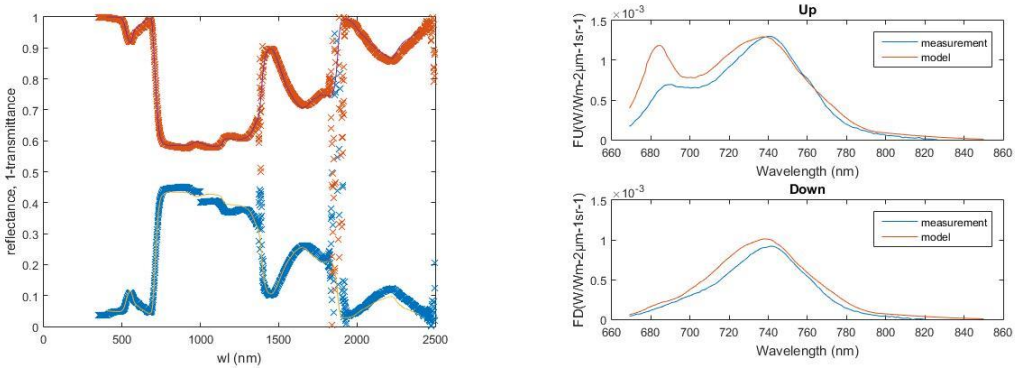
# 4. RESULTS

After each simulation, the simulated and measured reflectance and transmittance data plotted together for visual inspection. The upward and downward fluorescence of one leaf were also produced in one figure, comparing with the measured data. Here some representative leaves are shown for different natural conditions. The simulation results after tuning of the FQE parameters are presented as well.

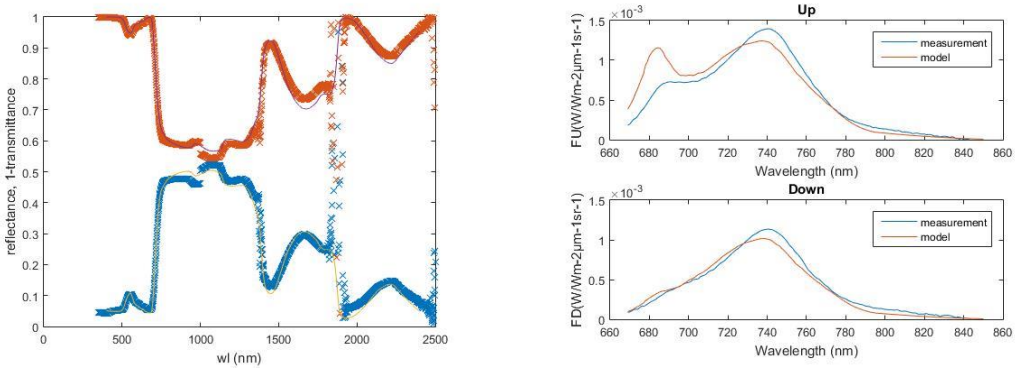
The reflectance and transmittance data were used to retrieve leaf composition and structure by using the RTM inversion model. The model's target is to calibrate the R and T to find the vegetation parameters that give the best match of simulated and measured R and T. With the vegetation parameters, the chlorophyll fluorescence can be simulated by Fluspect model. The simulation fluorescence up and down curves will be calculated and produced in the figure. The total fluorescence contains the upward and downward fluorescence. It will be shown as two curves from 680 to 800 nm wavelength and compared with each measured curve. Normally, the fluorescence will show two peaks which will appear at 687 and 740 nm. For the high chlorophyll content leaves, the first peaks in fluorescence down curves are not easy to identify in actual measurement, because the chlorophyll will absorb some of the produced fluorescence. So, for the first peak in the fluorescence down, it will be notable in the low chlorophyll content leaves.

## 4.1. Simulation results by Fluspect model

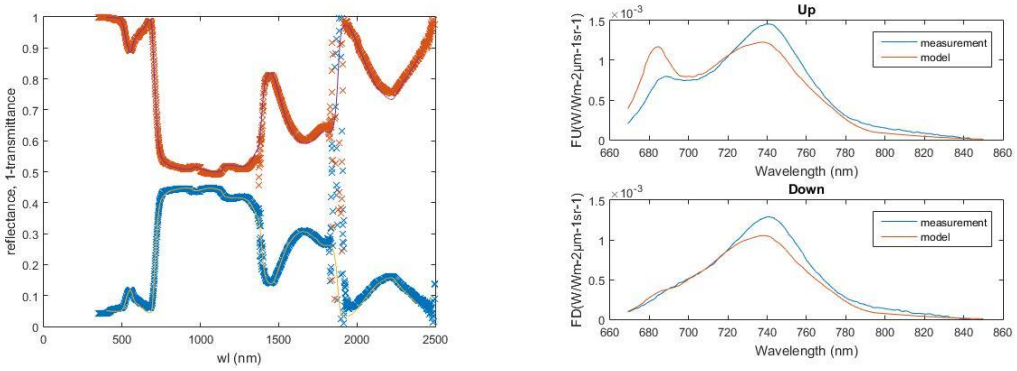
### 4.1.1. The simulation fluorescence for the high chlorophyll content leaves



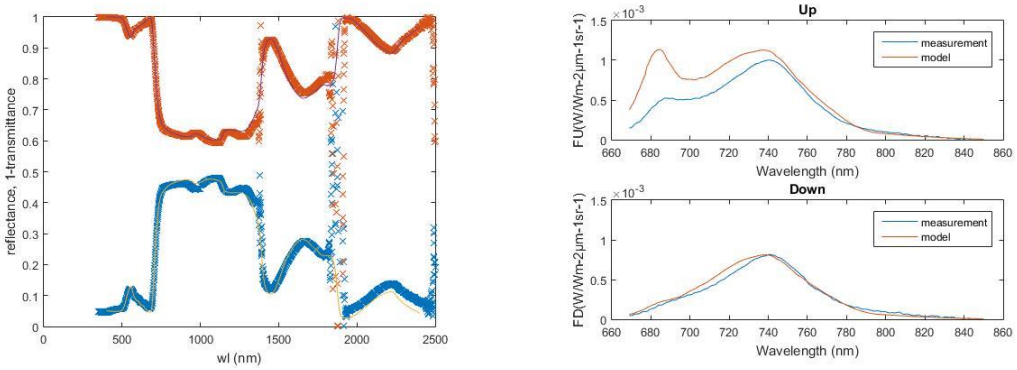

A




B




C




D

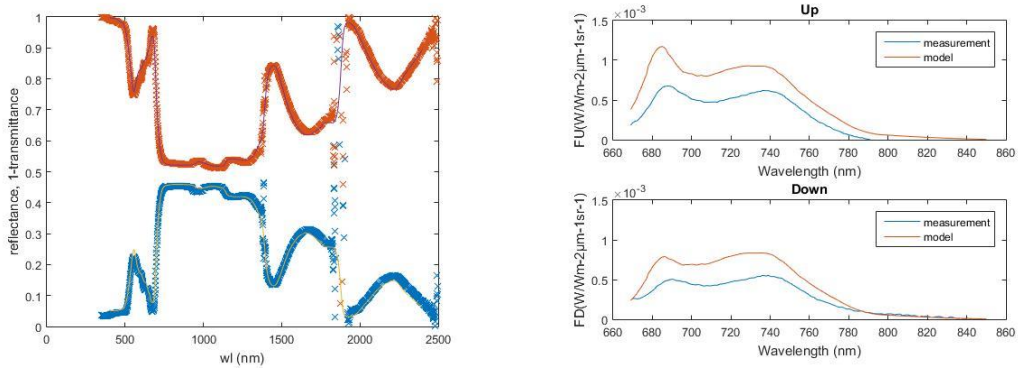
Figure 4- 1 The simulation results for the high chlorophyll content leaves

Table 4- 1 The simulation parameters for the high chlorophyll content leaves

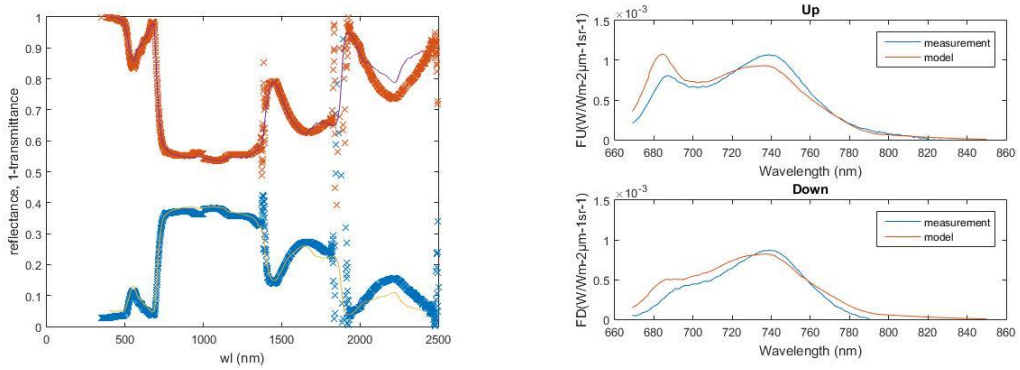
| Leaf | A | B | C | D |
|---|---|---|---|---|
| Cab (µg cm-2) | 53.90 | 56.59 | 41.15 | 43.40 |
| Cw (mg cm-2) | 0.02 | 0.02 | 0.01 | 0.02 |
| Cdm (mg cm-2) | 0.02 | 0.01 | 0.00 | 0.01 |
| Cs (fraction) | 0.00 | 0.46 | 0.24 | 0.41 |
| Cca (µg cm-2) | 9.77 | 7.94 | 7.07 | 11.49 |
| N (dimensionless) | 1.64 | 1.85 | 1.44 | 1.88 |
| RMSE (mod-meas spectra) | 0.60 | 0.69 | 0.38 | 0.56 |

As shown in Figure 4- 1, the simulation for the high chlorophyll content leaves is always overestimated, especially for the fluorescence up in the first peak in 687 nm, while for the second peak, the model performs relatively well. The simulations for the fluorescence down, the results are quite good. The simulation for the reflectance and the transmittance simulation is very good comparing with the measured data. Only the first peak of the fluorescence up is always overestimated. So the model performs relatively well in the fluorescence simulations for the high chlorophyll content leaves.

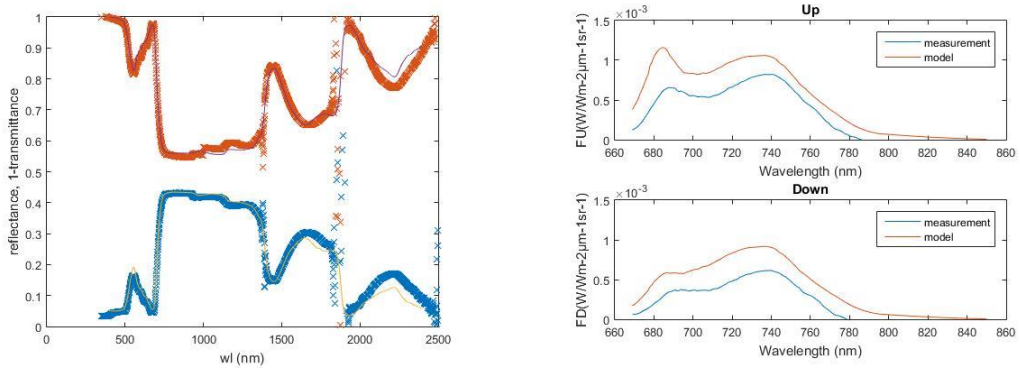
### 4.1.2. The simulation fluorescence for the low chlorophyll content leaves



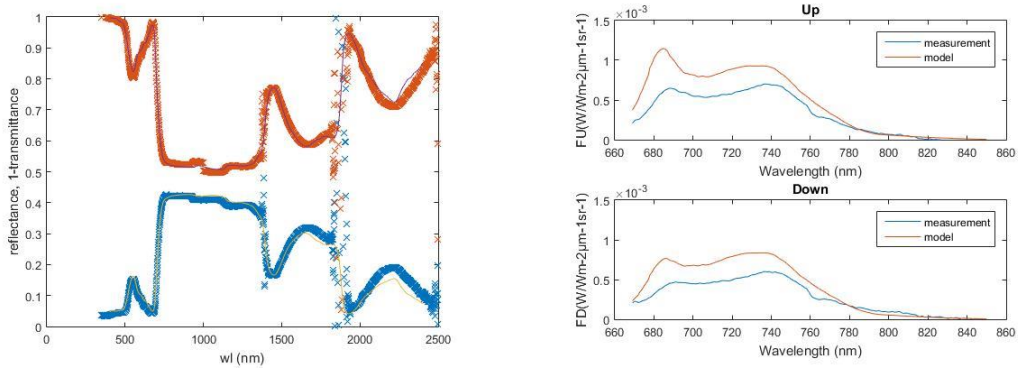

A




B




C




D

Figure 4- 2 The simulation results for the low chlorophyll content leaves

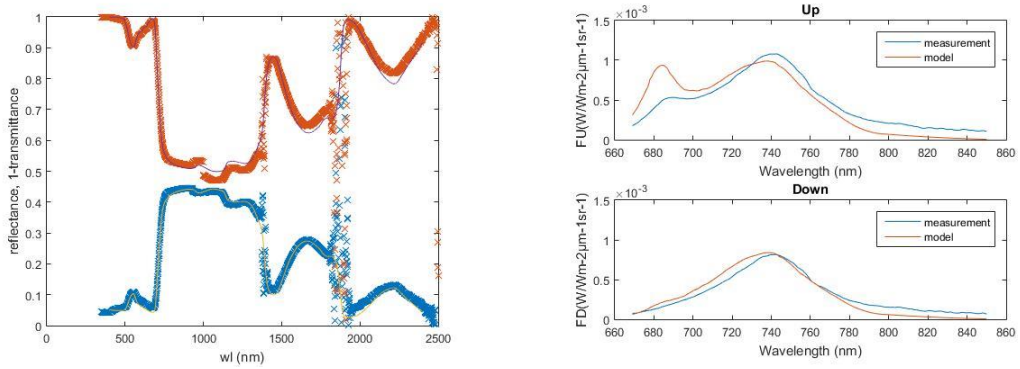
Table 4- 2 The simulation parameters for the low chlorophyll content leaves

| Leaf | A | B | C | D |
|---|---|---|---|---|
| Cab (µg cm-2) | 15.92 | 29.26 | 25.40 | 26.79 |
| Cw (mg cm-2) | 0.02 | 0.01 | 0.01 | 0.01 |
| Cdm (mg cm-2) | 0.01 | 0.02 | 0.01 | 0.01 |
| Cs (fraction) | 0.02 | 0.28 | 0.00 | 0.11 |
| Cca (µg cm-2) | 8.35 | 6.16 | 7.47 | 4.81 |
| N (dimensionless) | 1.51 | 1.37 | 1.55 | 1.40 |
| RMSE (mod-meas spectra) | 0.46 | 0.58 | 0.52 | 0.51 |

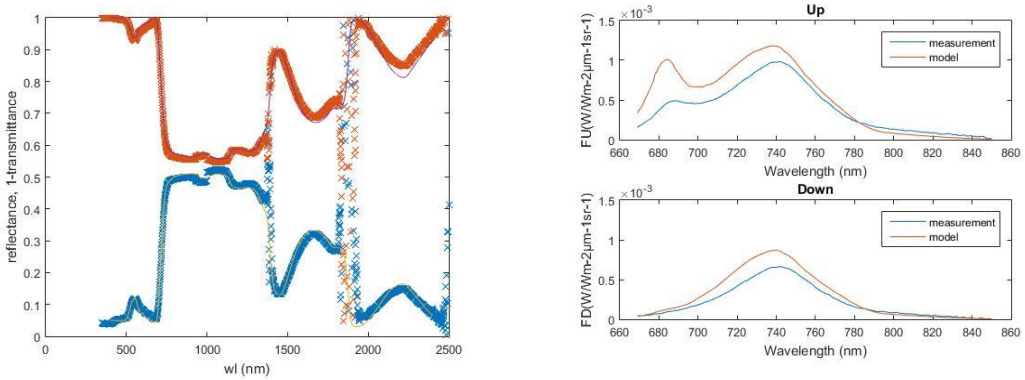
For the low chlorophyll content leaves fluorescence simulation, mostly reflectance and transmittance are simulated very well by Fluspect model. The fluorescence up and down are overestimated in the leaf a, c and d. For these three leaves, the 1st and 2nd peaks of fluorescence are overestimated in upward and downward direction. Only the simulation for the leaf b is underestimated in the 2nd peak in upward and downward. In general, the simulation fluorescence for low chlorophyll content leaves is overestimated in whole wavelength. Some simulations as Figure 4- 2 b will underestimated in the 2nd peak in up and down fluorescence. The photosystem plays a role in the peaks of fluorescence and also have the influence on the fluorescence in whole wavelength. The fluorescence will produce more with larger values of FQE II and I especially in the 1st and 2nd peaks of fluorescence curve. It is necessary to adjust the default FQE values to get a better agreement of simulated fluorescence to measurements. It can be concluded that the

simulations of the fluorescence are always overestimated by the Fluspect model for the low chlorophyll content leaves.

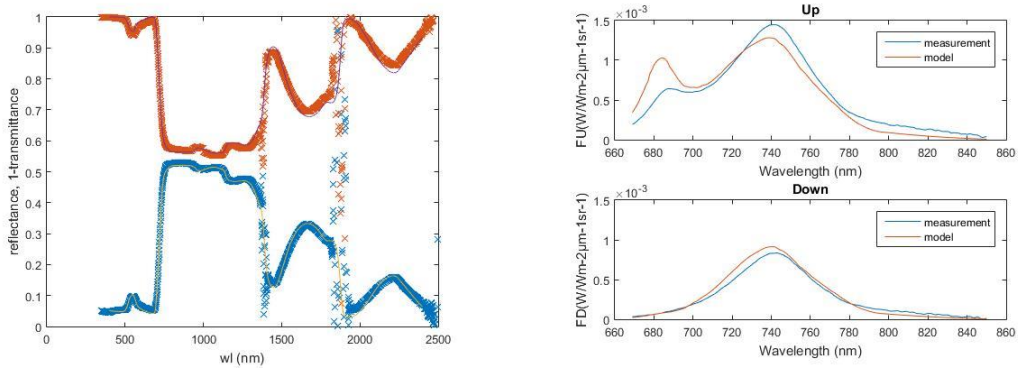
### 4.1.3. The simulation fluorescence for the high chlorophyll content leaves on both top and middle



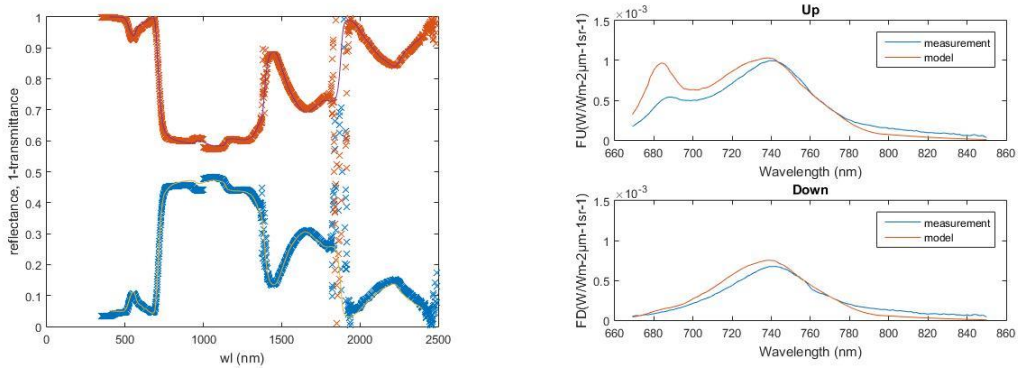

A




B




C

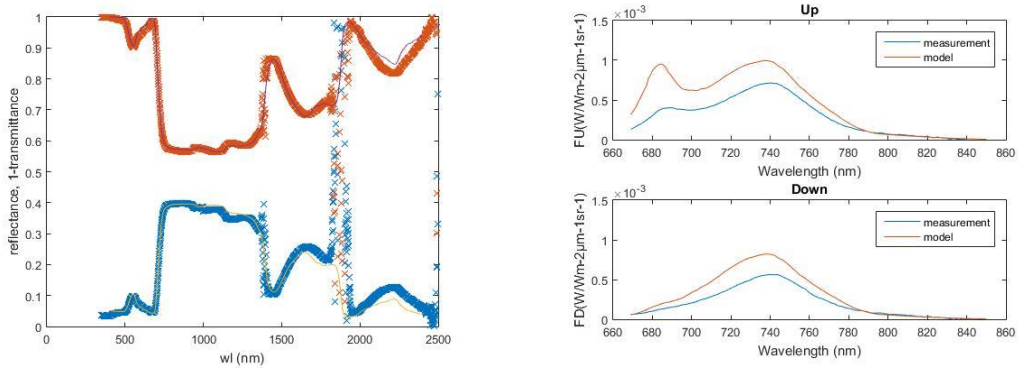


D

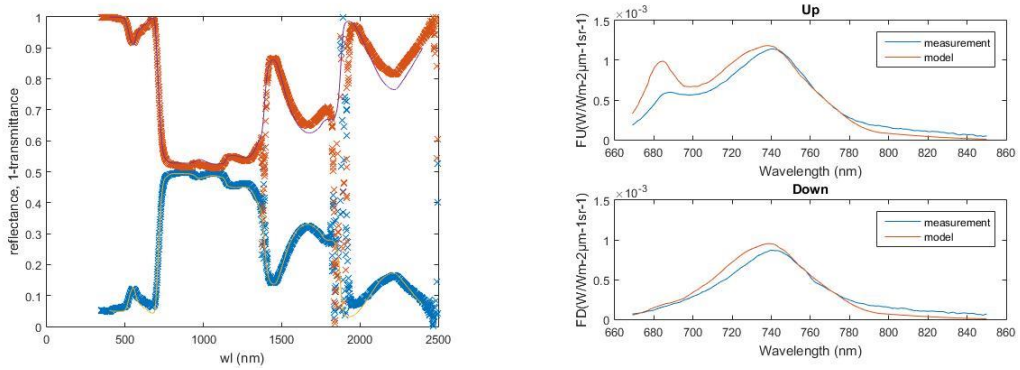
Figure 4- 3 The simulation results for the high chlorophyll content leaves on the top

Table 4- 3 The simulation parameters for the high chlorophyll content leaveson the top

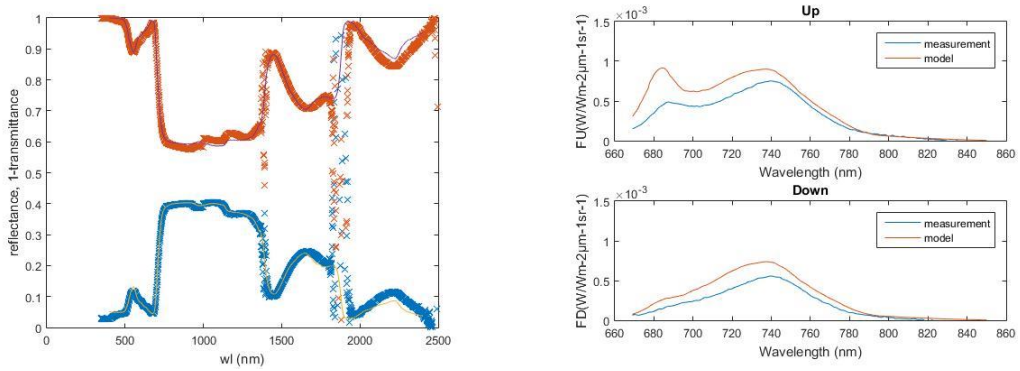
| Leaf | A | B | C | D |
|---|---|---|---|---|
| Cab (µg cm-2) | 46.43 | 55.65 | 75.19 | 53.69 |
| Cw (mg cm-2) | 0.02 | 0.02 | 0.02 | 0.02 |
| Cdm (mg cm-2) | 0.005 | 0.004 | 0.003 | 0.011 |
| Cs (fraction) | 0.32 | 0.19 | 0.05 | 0.32 |
| Cca (µg cm-2) | 6.35 | 7.47 | 6.67 | 9.07 |
| N (dimensionless) | 1.43 | 1.82 | 1.86 | 1.79 |
| RMSE (mod-meas spectra) | 0.67 | 0.82 | 0.62 | 0.43 |



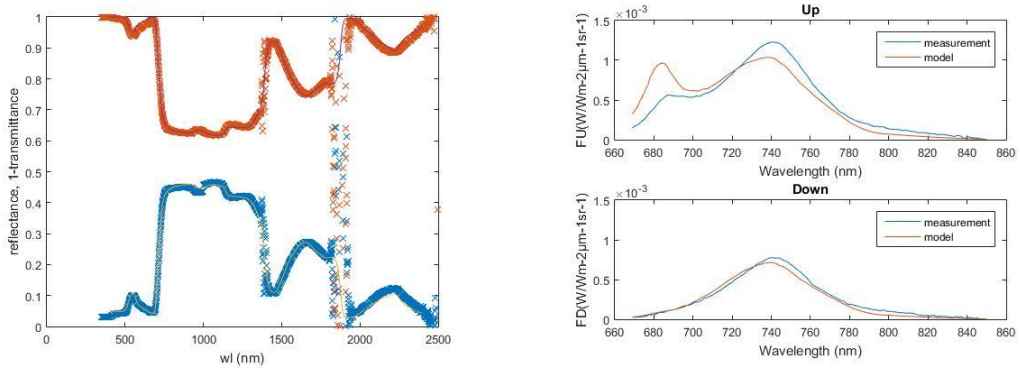

A




B



C




D

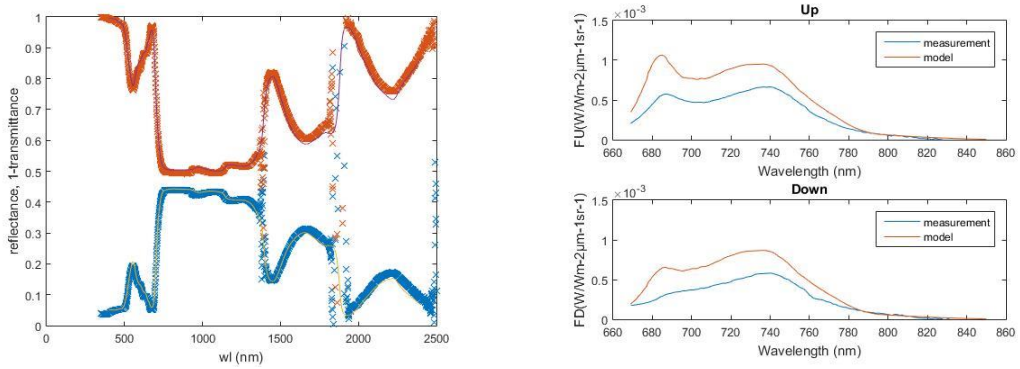
Figure 4- 4 The simulation results for the high chlorophyll content leaves on the middle

Table 4- 4 The simulation parameters for the high chlorophyll content leaves on the middle

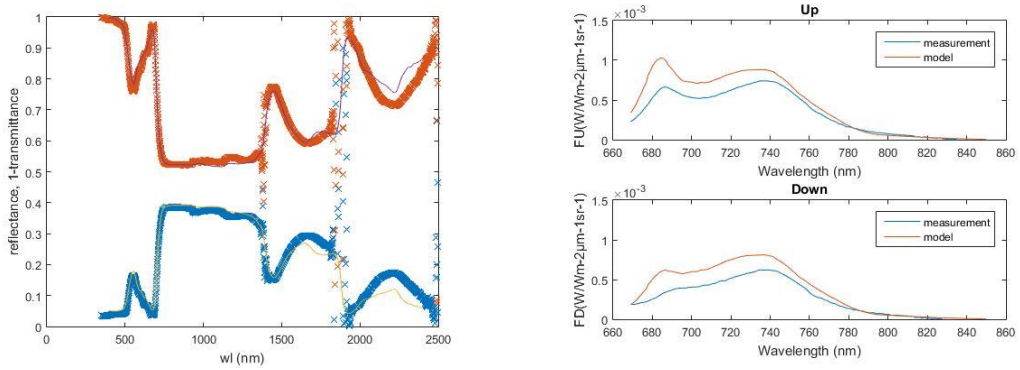
| Leaf | A | B | C | D |
|---|---|---|---|---|
| Cab (μg cm-2) | 50.46 | 51.44 | 38.33 | 61.44 |
| Cw (mg cm-2) | 0.02 | 0.02 | 0.01 | 0.02 |
| Cdm (mg cm-2) | 0.02 | 0.003 | 0.02 | 0.02 |
| Cs (fraction) | 0.08 | 0.03 | 0.12 | 0.26 |
| Cca (μg cm-2) | 8.63 | 8.01 | 8.34 | 7.68 |
| N (dimensionless) | 1.47 | 1.64 | 1.56 | 1.87 |
| RMSE (mod-meas spectra) | 0.54 | 0.93 | 0.61 | 0.45 |

After comparing the parameters and the fluorescence for the high chlorophyll content leaves on the top and middle of the plants, the simulation results are almost same (see Figure 4- 4). The simulation results are higher than the observed data especially in the first peak in fluorescence up. In some condition, the model underestimated fluorescence on the second peak in both fluorescence up and down. The situation may happen due to the weather condition. The main factor PS II and I will vary that will produce more fluorescence in actual condition. That means the default FQE values are not enough to do an accurate simulation.

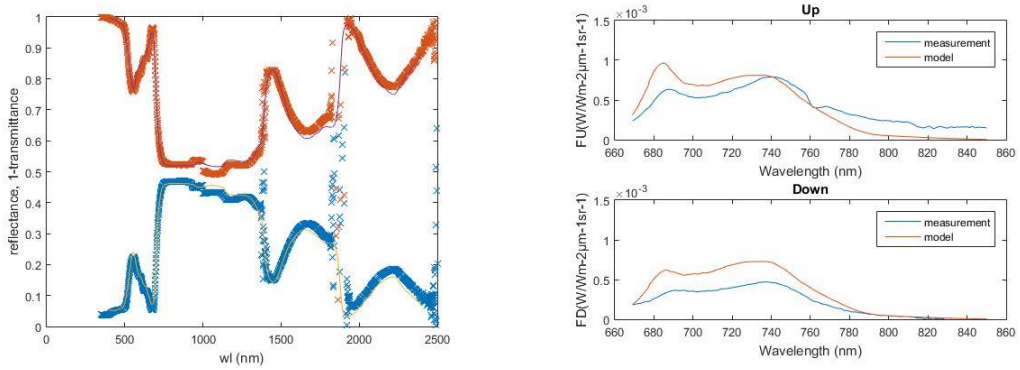
### 4.1.4. The simulation fluorescence for the low chlorophyll content leaves on both top and middle



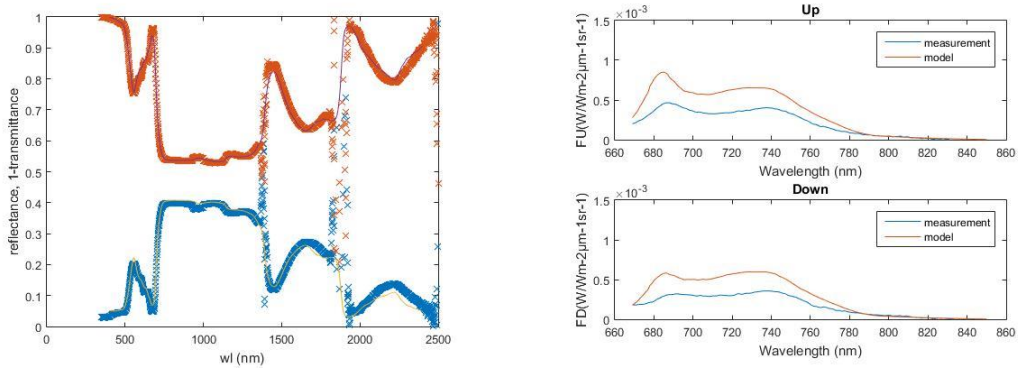

A




B




C

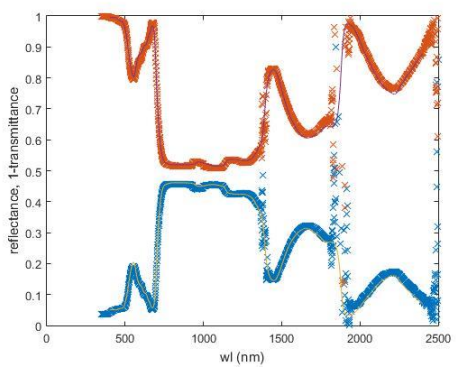


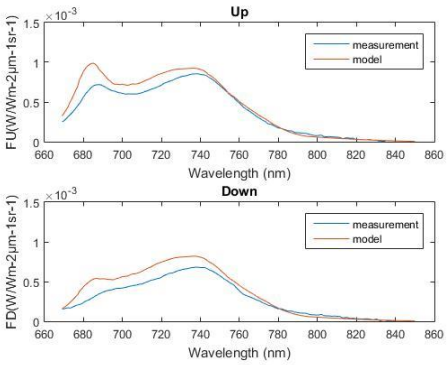

D

Figure 4- 5 The simulation results for the low chlorophyll content leaves on the top

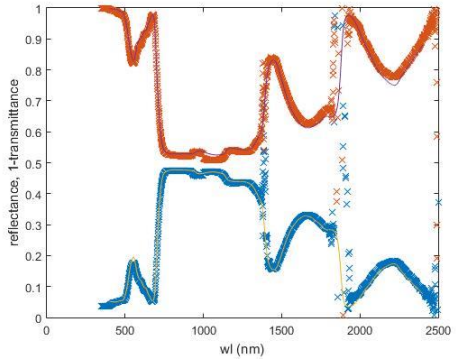
Table 4- 5 The simulation parameters for the low chlorophyll content leaves on the top

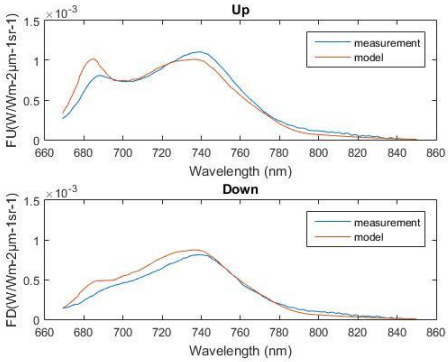
| Leaf | A | B | C | D |
|---|---|---|---|---|
| Cab (µg cm-2) | 20.96 | 22.26 | 17.74 | 16.63 |
| Cw (mg cm-2) | 0.01 | 0.01 | 0.01 | 0.01 |
| Cdm (mg cm-2) | 0.01 | 0.01 | 0.01 | 0.01 |
| Cs (fraction) | 0 | 0.014 | 0 | 0 |
| Cca (µg cm-2) | 6.02 | 5.25 | 6.03 | 7.57 |
| N (dimensionless) | 1.42 | 1.32 | 1.52 | 1.41 |
| RMSE (mod-meas spectra) | 0.75 | 0.64 | 0.68 | 0.79 |


reflectance, 1-transmittance
wl (nm)

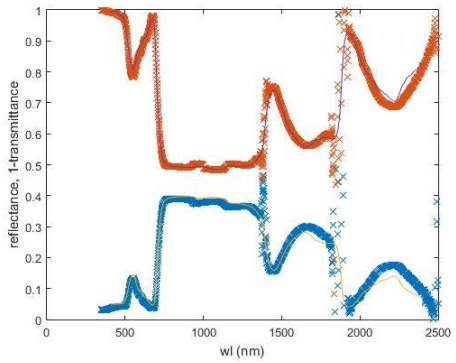

Up
measurement
model
FU(W/Wm-2μm-1sr-1)
Wavelength (nm)
Down
measurement
model
FD(W/Wm-2μm-1sr-1)
Wavelength (nm)

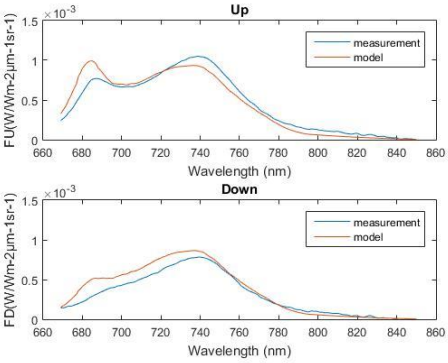
A


reflectance, 1-transmittance
wl (nm)

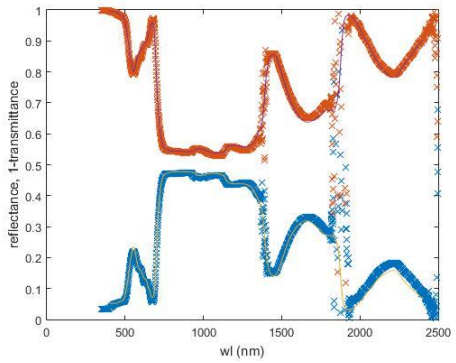

Up
measurement
model
FU(W/Wm-2μm-1sr-1)
Wavelength (nm)
Down
measurement
model
FD(W/Wm-2μm-1sr-1)
Wavelength (nm)

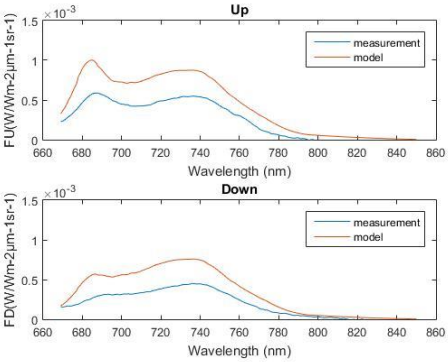
B


reflectance, 1-transmittance
wl (nm)

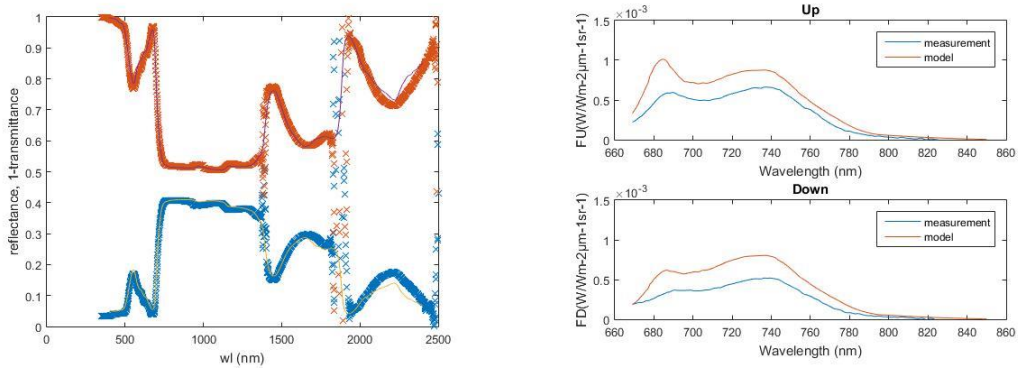

Up
measurement
model
FU(W/Wm-2μm-1sr-1)
Wavelength (nm)
Down
measurement
model
FD(W/Wm-2μm-1sr-1)
Wavelength (nm)

C


reflectance, 1-transmittance
wl (nm)


Up
measurement
model
FU(W/Wm-2μm-1sr-1)
Wavelength (nm)
Down
measurement
model
FD(W/Wm-2μm-1sr-1)
Wavelength (nm)

D




E

Figure 4- 6 The simulation results for the low chlorophyll content leaves on the middle

Table 4- 6 The simulation parameters for the low chlorophyll content leaves on the middle

| Leaf | A | B | C | D | E |
|---|---|---|---|---|---|
| Cab (µg cm-2) | 23.18 | 27.04 | 28.35 | 19.23 | 21.70 |
| Cw (mg cm-2) | 0.01 | 0.01 | 0.01 | 0.02 | 0.01 |
| Cdm (mg cm-2) | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Cs (fraction) | 0.02 | 0.01 | 0.08 | 0.05 | 0.05 |
| Cca (µg cm-2) | 5.52 | 5.70 | 4.55 | 5.92 | 5.59 |
| N (dimensionless) | 1.51 | 1.59 | 1.26 | 1.64 | 1.35 |
| RMSE (mod-meas spectra) | 0.44 | 0.75 | 0.58 | 0.52 | 0.61 |

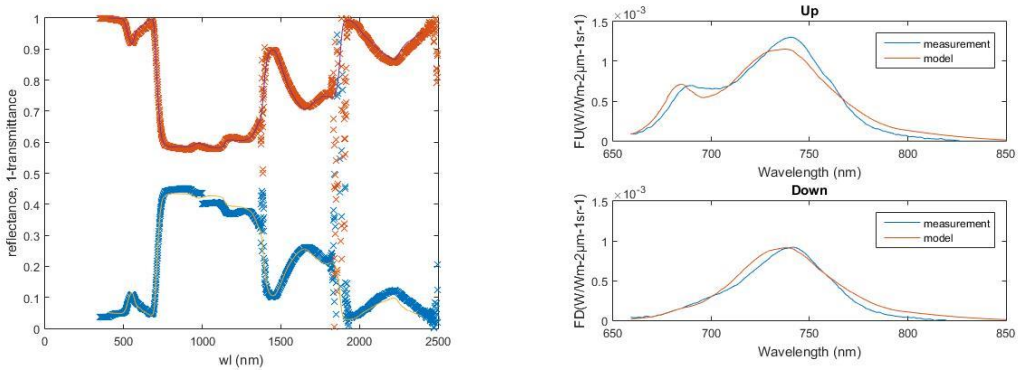
The situation for the low chlorophyll content leaves still overestimated the fluorescence. The simulation results for the leaves on the top and bottom are both overestimated. The simulation results of upward and downward fluorescence are overestimated. It can be concluded that the simulation for two different leaves and the position on the plant have little influence on the simulation. The model always overestimates the fluorescence for different leaves.

For the all simulations, the simulated fluorescence is overestimated. For the high chlorophyll content leaves, the simulated fluorescence up is higher than the measured data, especially in the first peak in 687nm. The simulation for the fluorescence up in the second peak is better than the simulation results in the first peak. For the low chlorophyll content leaves, the simulation fluorescence is always overestimated especially around the 740nm in both fluorescence up and down. Further analysis is presented in the next section.

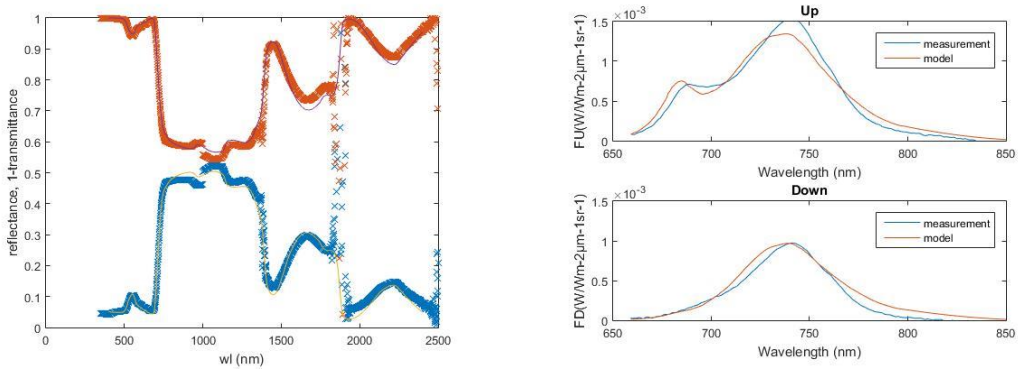
## 4.2. Simulation results by Fluspect model with adding FQE inversion code

The simulation results show that the results are always overestimated especially in the first peak and the second peak. The photosystem II and I have a great influence on the emission of these parts. The FQE value is as a basic in the model simulation. So the improvement of the Fluspect model is adding the FQE inversion code. With the same measured data, the simulation was done again to get new simulation results.

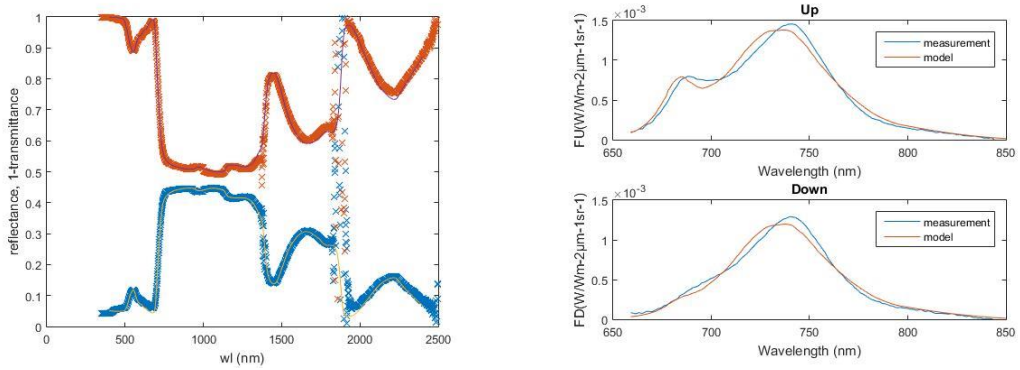
### 4.2.1. The simulation fluorescence for the different chlorophyll content leaves



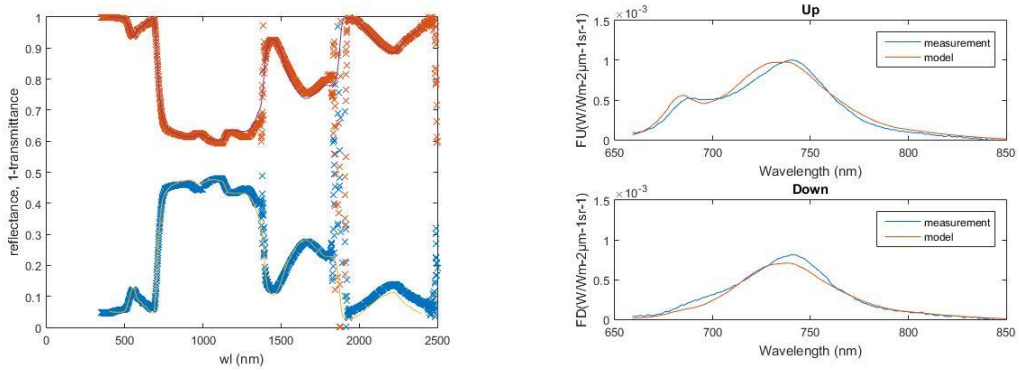

A




B




C

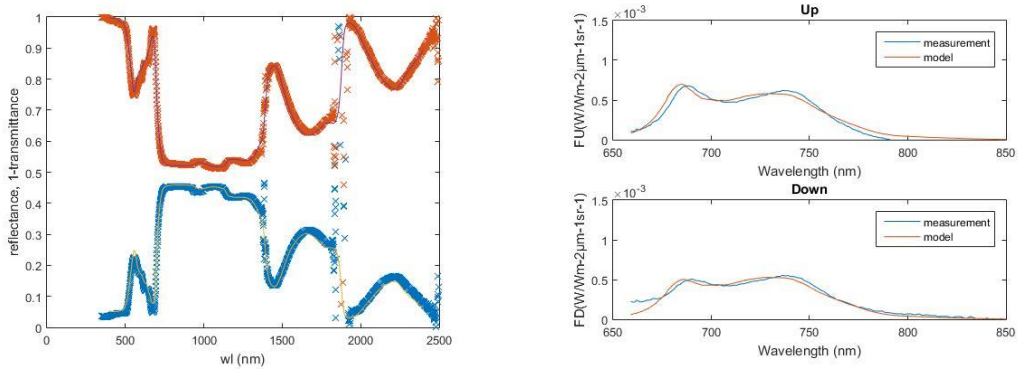



D

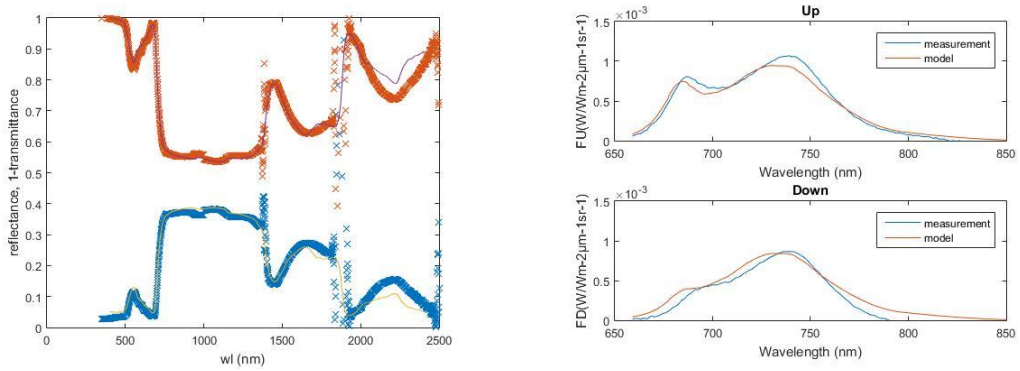
Figure 4- 7 The simulation results with FQE inversion for the high chlorophyll content leaves

Table 4- 7 The simulation parameters with FQE inversion for the high chlorophyll content leaves

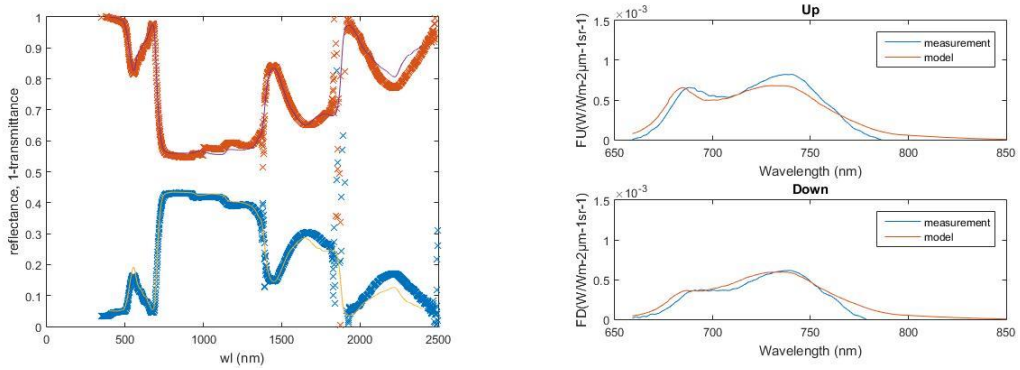
| Leaf | A | B | C | D |
|---|---|---|---|---|
| Cab (µg cm-2) | 53.91 | 56.59 | 41.12 | 43.40 |
| Cw (mg cm-2) | 0.02 | 0.02 | 0.01 | 0.02 |
| Cdm (mg cm-2) | 0.02 | 0.01 | 0.00 | 0.01 |
| Cs (fraction) | 0.00 | 0.46 | 0.24 | 0.41 |
| Cca (µg cm-2) | 9.56 | 7.75 | 6.72 | 11.11 |
| N (dimensionless) | 1.64 | 1.85 | 1.44 | 1.88 |
| FQE1 (dimensionless) | 0.003 | 0.004 | 0.004 | 0.004 |
| FQE2 (dimensionless) | 0.006 | 0.006 | 0.006 | 0.005 |
| RMSE (mod-meas spectra) | 53.91 | 56.59 | 41.12 | 43.40 |



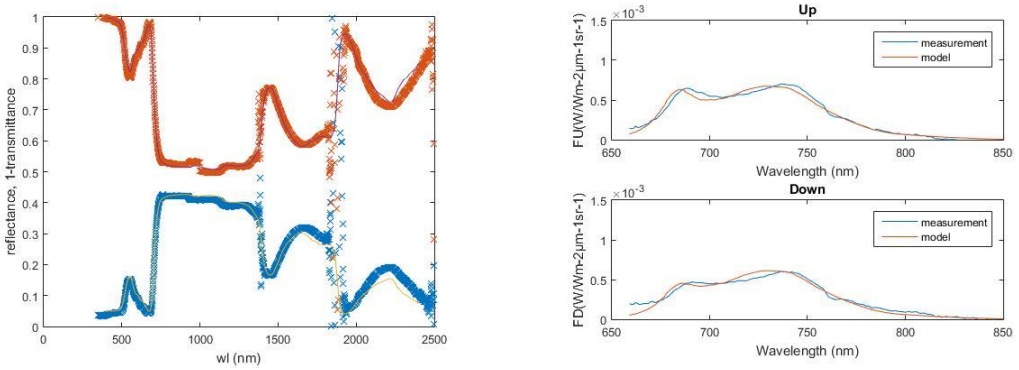

A




B




C




D

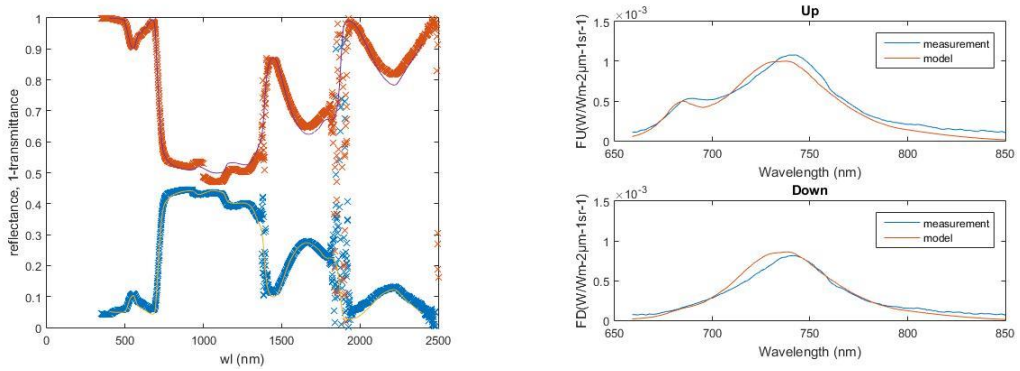
Figure 4- 8 The simulation results with FQE inversion for the low chlorophyll content leaves

Table 4- 8 The simulation parameters with FQE inversion for the low chlorophyll content leaves

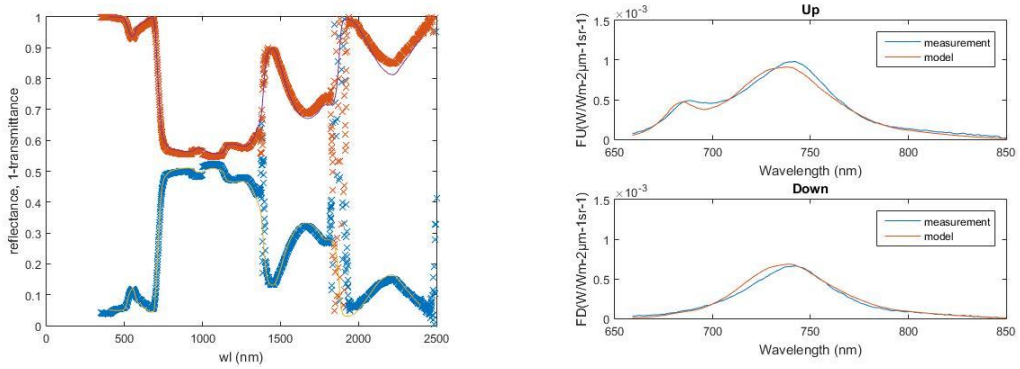
| Leaf | A | B | C | D |
|---|---|---|---|---|
| Cab (μg cm-2) | 15.92 | 29.29 | 25.39 | 26.82 |
| Cw (mg cm-2) | 0.02 | 0.01 | 0.01 | 0.01 |
| Cdm (mg cm-2) | 0.01 | 0.02 | 0.01 | 0.01 |
| Cs (fraction) | 0.02 | 0.28 | 0.00 | 0.11 |
| Cca (μg cm-2) | 8.37 | 6.35 | 7.47 | 4.96 |
| N (dimensionless) | 1.51 | 1.37 | 1.55 | 1.40 |
| FQE1 (dimensionless) | 0.002 | 0.003 | 0.002 | 0.004 |
| FQE2 (dimensionless) | 0.006 | 0.006 | 0.005 | 0.010 |
| RMSE (mod-meas spectra) | 0.45 | 0.57 | 0.50 | 0.49 |

The photosystem II and I were given a value after adding FQE inversion code. These values are smaller than the defaults which have been given as a basic in mode simulation. The simulated curves have been improved by adding the inversion code. From the Figure 4- 7 and Figure 4- 8 the simulation results show a good relationship with the measured data. The peaks of simulation results are close to de measured data. The vegetation parameters were also retrieved again. They did not change a lot by comparing the retrieved parameters in Table 4- 1. Because the parameters without FQE are retrieved by the reflectance and transmittance data, the FQE values are retrieved by the measured fluorescence to get more accurate simulation. The improvement of the simulation results means the FQE values are most important in simulating the fluorescence.

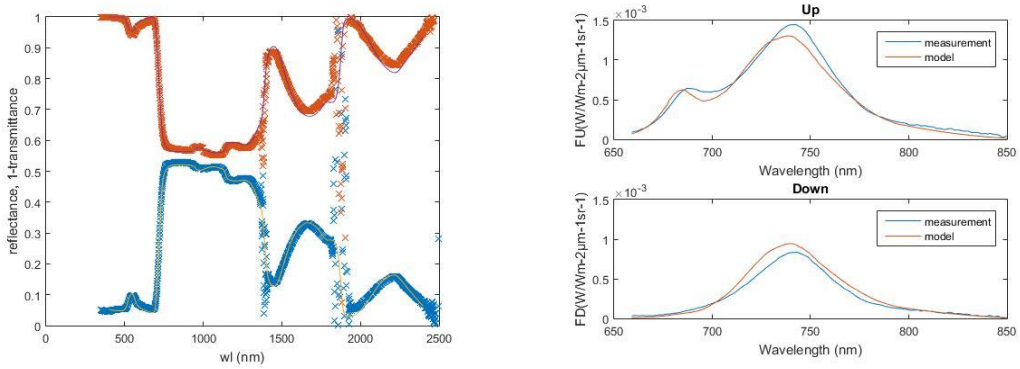
### 4.2.2. The simulation fluorescence for the high chlorophyll content leaves on both top and middle



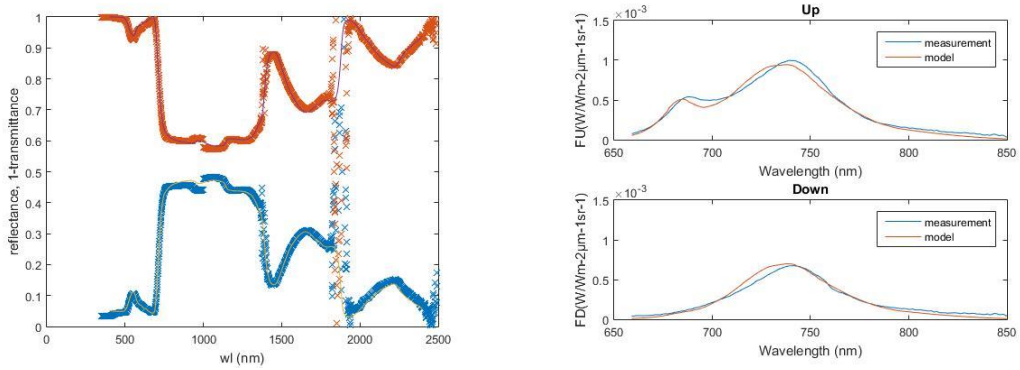

A




B




C

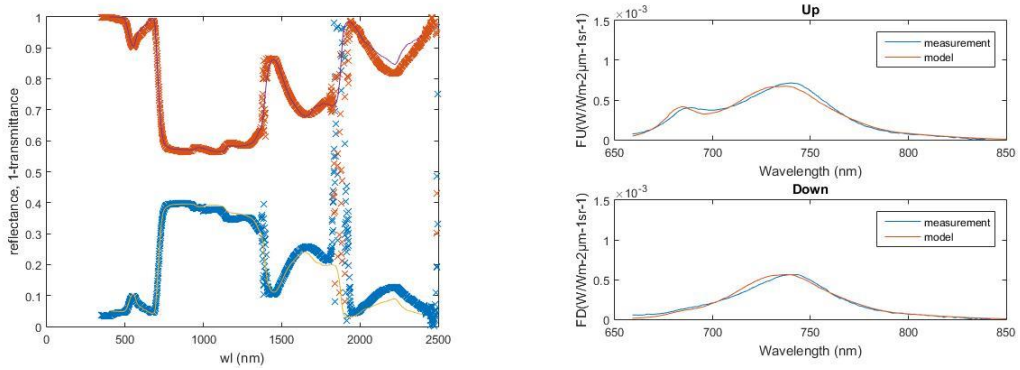



D

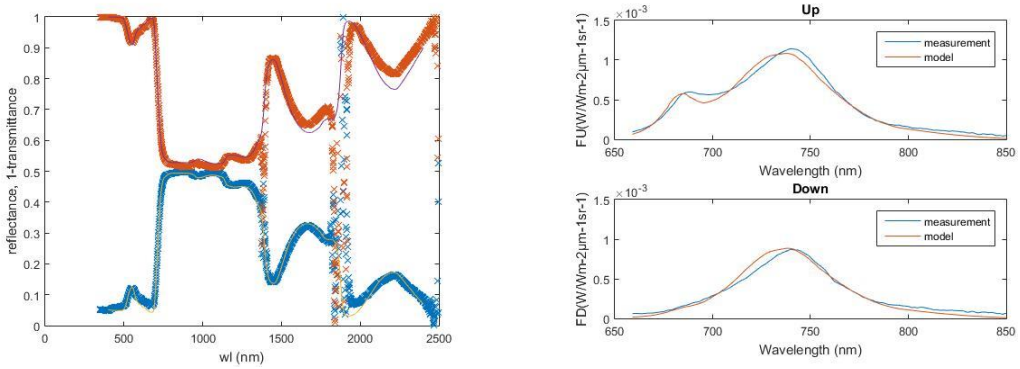
Figure 4- 9 The simulation results with FQE inversion for the high chlorophyll content leaves on the top

Table 4- 9 The simulation parameters with FQE inversion for the high chlorophyll content leaves on the top

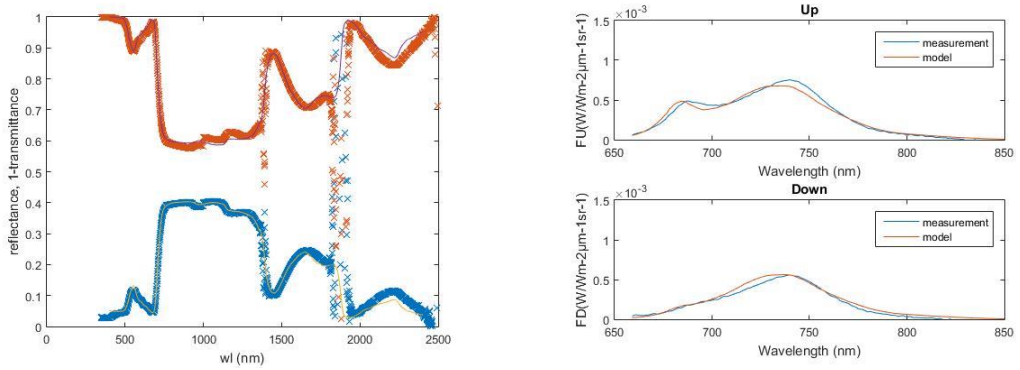
| Leaf | A | B | C | D |
|---|---|---|---|---|
| Cab (µg cm-2) | 46.40 | 55.63 | 75.19 | 53.69 |
| Cw (mg cm-2) | 0.02 | 0.02 | 0.02 | 0.02 |
| Cdm (mg cm-2) | 0.005 | 0.004 | 0.004 | 0.012 |
| Cs (fraction) | 0.33 | 0.19 | 0.05 | 0.32 |
| Cca (µg cm-2) | 5.99 | 7.21 | 6.47 | 9.07 |
| N (dimensionless) | 1.43 | 1.82 | 1.86 | 1.79 |
| FQE1 (dimensionless) | 0.004 | 0.003 | 0.004 | 0.004 |
| FQE2 (dimensionless) | 0.005 | 0.004 | 0.005 | 0.005 |
| RMSE (mod-meas spectra) | 46.40 | 55.63 | 75.19 | 53.69 |



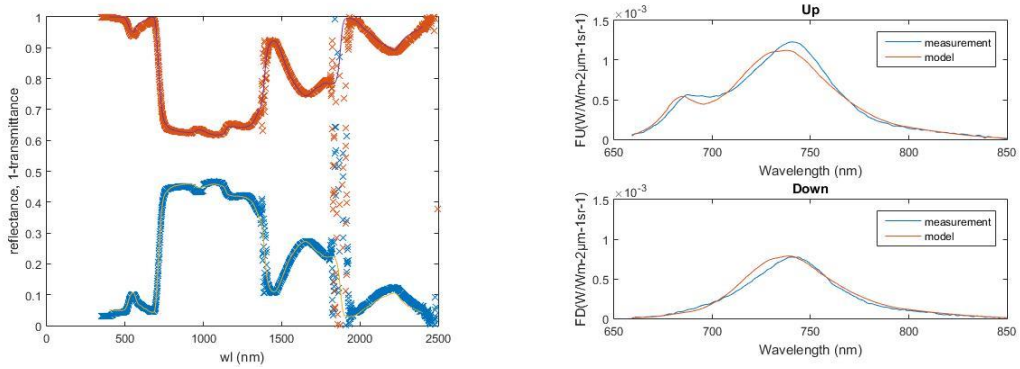

A




B




C




D

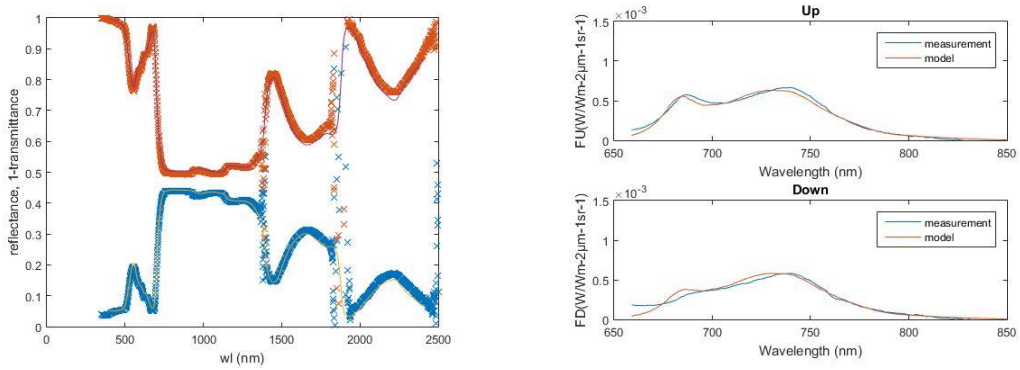
Figure 4- 10 The simulation results with FQE inversion for the high chlorophyll content leaves on the middle

Table 4- 10 The simulation parameters with FQE inversion for the high chlorophyll content leaves on the middle

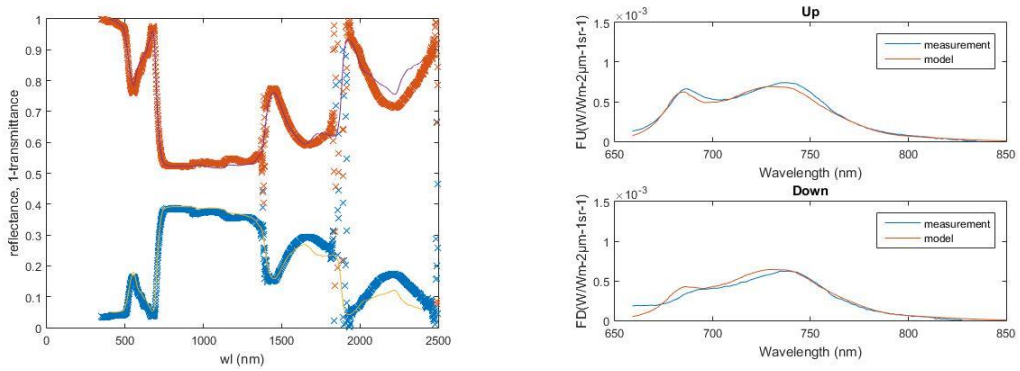
| Leaf | A | B | C | D |
|---|---|---|---|---|
| Cab (µg cm-2) | 50.45 | 51.43 | 38.32 | 61.45 |
| Cw (mg cm-2) | 0.02 | 0.02 | 0.02 | 0.02 |
| Cdm (mg cm-2) | 0.02 | 0.00 | 0.02 | 0.02 |
| Cs (fraction) | 0.08 | 0.03 | 0.13 | 0.26 |
| Cca (µg cm-2) | 8.34 | 7.65 | 8.16 | 7.69 |
| N (dimensionless) | 1.47 | 1.64 | 1.56 | 1.87 |
| FQE1 (dimensionless) | 0.003 | 0.003 | 0.003 | 0.004 |
| FQE2 (dimensionless) | 0.004 | 0.005 | 0.005 | 0.005 |
| RMSE (mod-meas spectra) | 0.52 | 0.90 | 0.60 | 0.44 |

The simulation fluorescence with the FQE inversion value is better than the results with default FQE values. In the FQE inversion, the FQE I value for the high chlorophyll content leaves on the top is higher than the leaves on the middle level of the plants. The difference is very small. For the FQE II the results are almost the same.

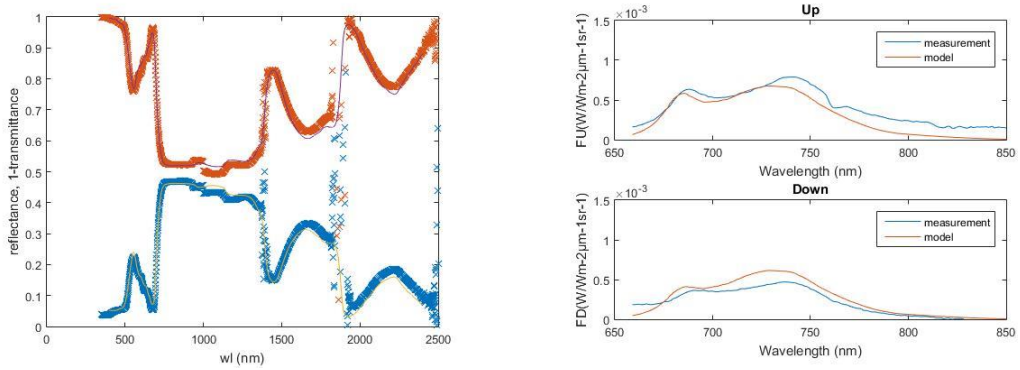
### 4.2.3. The simulation fluorescence for the low chlorophyll content leaves on both top and middle



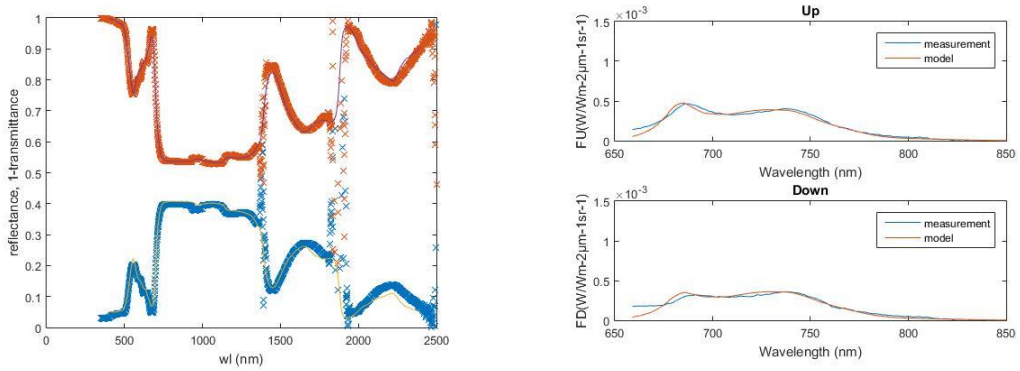

A




B




C

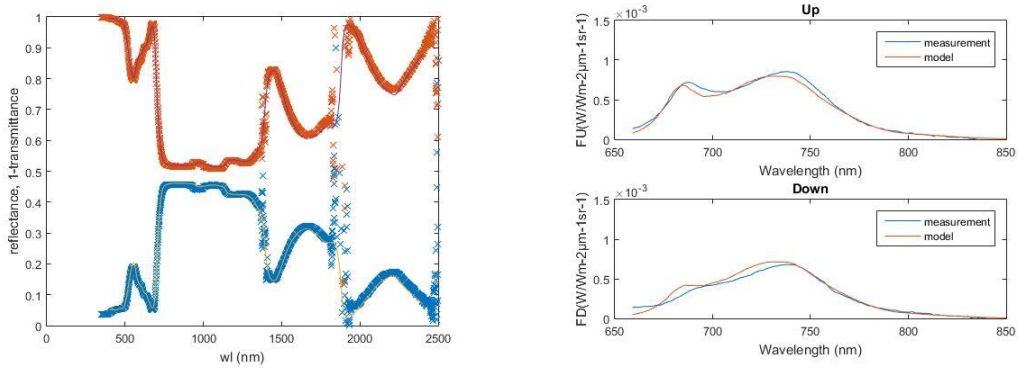



D

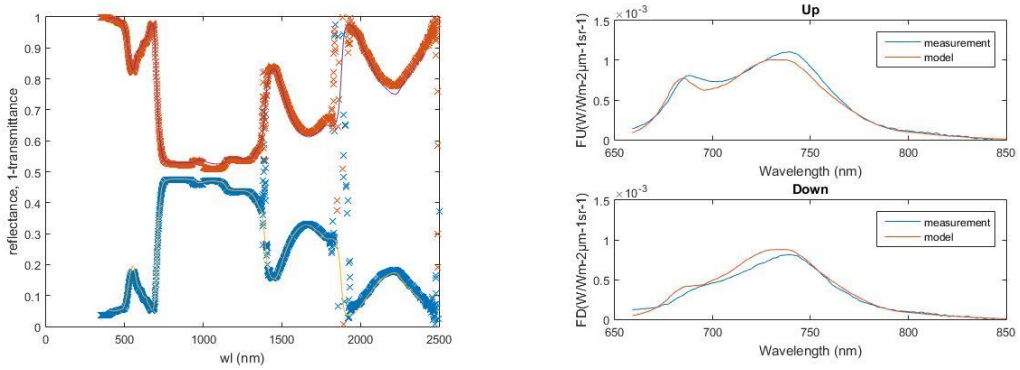
Figure 4- 11 The simulation results with FQE inversion for the low chlorophyll content leaves on the top

Table 4- 11 The simulation parameters with FQE inversion for the low chlorophyll content leaves on the top

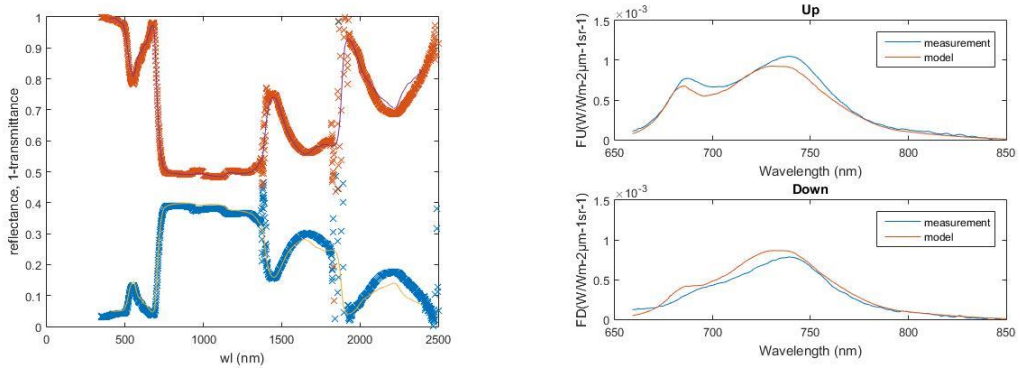
| Leaf | A | B | C | D |
|---|---|---|---|---|
| Cab (µg cm-2) | 20.96 | 22.29 | 17.73 | 16.62 |
| Cw (mg cm-2) | 0.01 | 0.01 | 0.01 | 0.01 |
| Cdm (mg cm-2) | 0.01 | 0.02 | 0.01 | 0.01 |
| Cs (fraction) | 0 | 0.012 | 0 | 0 |
| Cca (µg cm-2) | 6.02 | 5.42 | 6.13 | 7.69 |
| N (dimensionless) | 1.42 | 1.32 | 1.52 | 1.41 |
| FQE1 (dimensionless) | 0.002 | 0.002 | 0.003 | 0.002 |
| FQE2 (dimensionless) | 0.005 | 0.005 | 0.005 | 0.005 |
| RMSE (mod-meas spectra) | 0.73 | 0.63 | 0.66 | 0.76 |



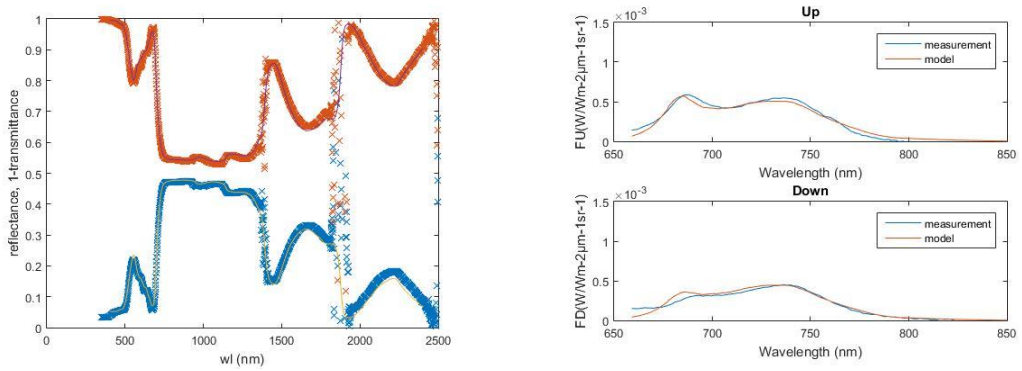

A



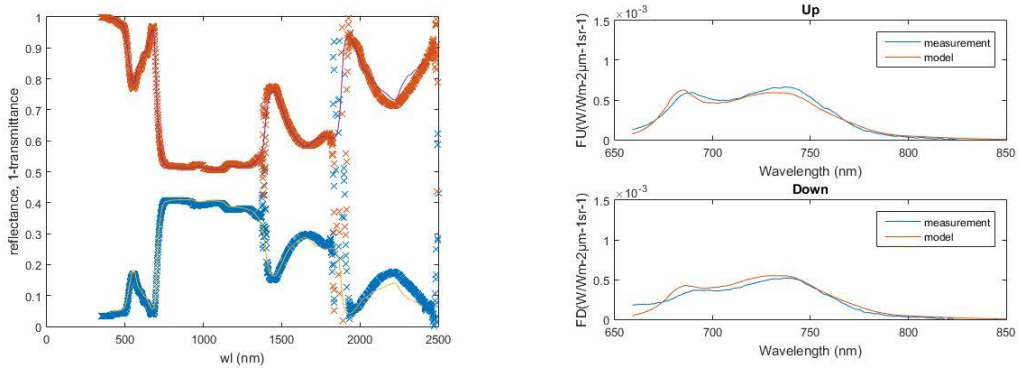

B




C




D




E

Figure 4- 12 The simulation results with FQE inversion for the low chlorophyll content leaves on the middle

Table 4- 12 The simulation parameters with FQE inversion for the low chlorophyll content leaves on the middle

| Leaf | A | B | C | D | E |
|---|---|---|---|---|---|
| Cab (µg cm-2) | 23.18 | 26.99 | 28.36 | 19.23 | 21.70 |
| Cw (mg cm-2) | 0.01 | 0.01 | 0.01 | 0.02 | 0.01 |
| Cdm (mg cm-2) | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Cs (fraction) | 0.02 | 0.01 | 0.08 | 0.04 | 0.05 |
| Cca (µg cm-2) | 5.56 | 5.54 | 4.63 | 6.00 | 5.64 |
| N (dimensionless) | 1.51 | 1.59 | 1.26 | 1.64 | 1.35 |
| FQE1 (dimensionless) | 0.002 | 0.003 | 0.003 | 0.001 | 0.002 |
| FQE2 (dimensionless) | 0.006 | 0.007 | 0.006 | 0.005 | 0.006 |
| RMSE (mod-meas spectra) | 0.42 | 0.72 | 0.57 | 0.50 | 0.59 |

After inversion the FQE II and I, the value has big difference than the default value. The he FQE II decrease to 0.006 from the 0.01. FQE II has not a big change. But the results with FQE inversion also improved. The FQE II and I have not a big difference on different levels of the plants.

# 5. DATA ANALYSES

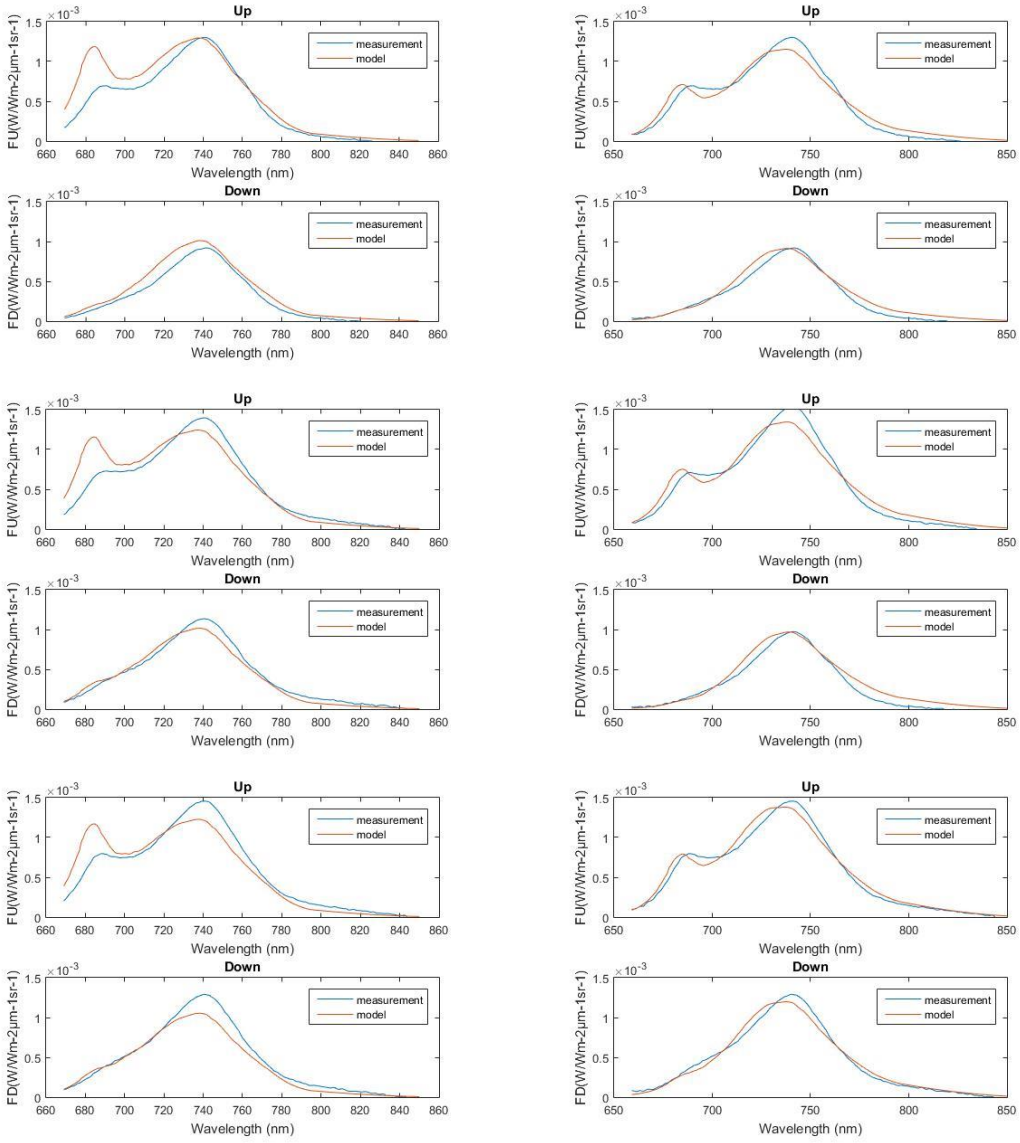
To assess the performance of the model efficiency, the simulation fluorescence and measured data have been put into one figure (show in Figure 5- 1). The results with the FQE inversion are also put together to evaluate the model.

The normalized PEARSON and R2 between independent in situ measurements and simulating fluorescence were used. We can compare the simulation fluorescence with the measured fluorescence in all wavelengths about the distribution of the curve. The peak values also can be compared to find the different between the simulation and measured fluorescence.

## 5.1. The results after model improvement

Simulation results

Simulation results with the FQE inversion code



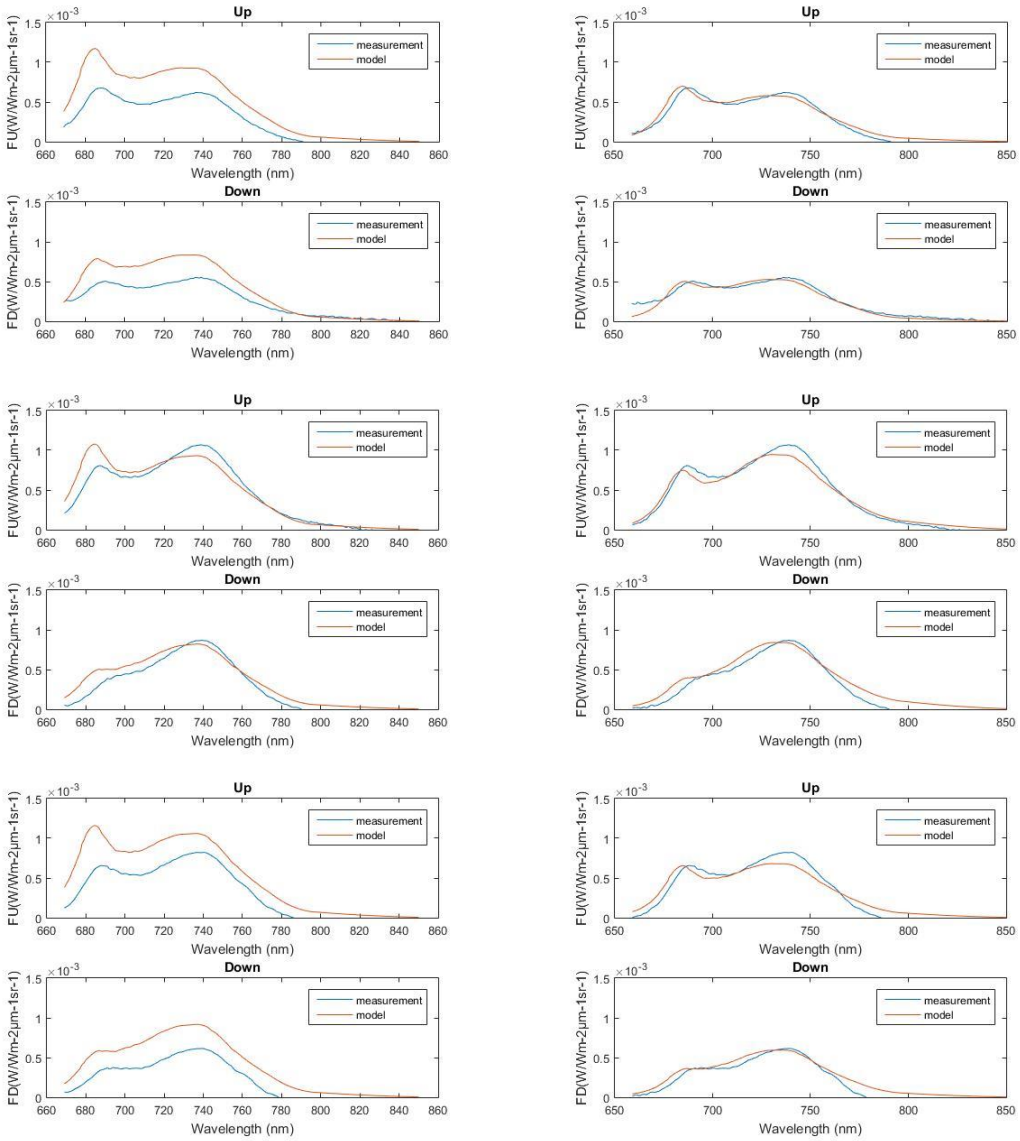



Figure 5- 1 The comparison between the original results and the results with FEQ inversion

Comparing two simulation results, it will be easy to see the simulation results are better than the simulation results without the FQE inversion code. The overestimated parts in the first peak and the second peak have been revised. That means the FQE II and I play an important role in the emission of the fluorescence in the first and the second peak. After tuning FQE to minimize the difference between measured and simulated fluorescence, the simulation results will be better.

## 5.2. PEARSON and $R^2$ index

Table 5- 1 shows the PEARSON and R2 index after comparing the simulation results with the measured data. The relationship between measured and simulated fluorescence is very good. For different leaves, the PEARSON indexes are above the 0.95 and the R2 is above 0.93. That means both simulated fluorescence up and down are fitted very well with the measured data. But these indexes only are on behalf of the correlation for all wavelengths together (650 to 850nm with 1 nm resolution. So in the whole wavelength, the model can perform very well. But from the figures it is easy to see, the model will overestimate in the first peak.

Table 5- 1 The relationship between measured and simulated fluorescence for six leaves

| INDEX | Fluorescence up for high chlorophyll content leaves | | | | | |
|---|---|---|---|---|---|---|
| PEARSON | 0.92 | 0.95 | 0.92 | 0.93 | 0.93 | 0.92 |
| $R^2$ | 0.84 | 0.90 | 0.85 | 0.86 | 0.87 | 0.84 |
| | Fluorescence down for high chlorophyll content leaves | | | | | |
| PEARSON | 0.98 | 0.99 | 0.99 | 0.98 | 0.98 | 0.99 |
| $R^2$ | 0.97 | 0.98 | 0.98 | 0.96 | 0.95 | 0.97 |
| | Fluorescence up for low chlorophyll content leaves | | | | | |
| PEARSON | 0.98 | 0.96 | 0.98 | 0.95 | 0.97 | 0.97 |
| $R^2$ | 0.97 | 0.93 | 0.96 | 0.90 | 0.94 | 0.94 |
| | Fluorescence down for low chlorophyll content leaves | | | | | |
| PEARSON | 0.96 | 0.97 | 0.98 | 0.97 | 0.99 | 0.98 |
| $R^2$ | 0.93 | 0.93 | 0.96 | 0.94 | 0.98 | 0.96 |

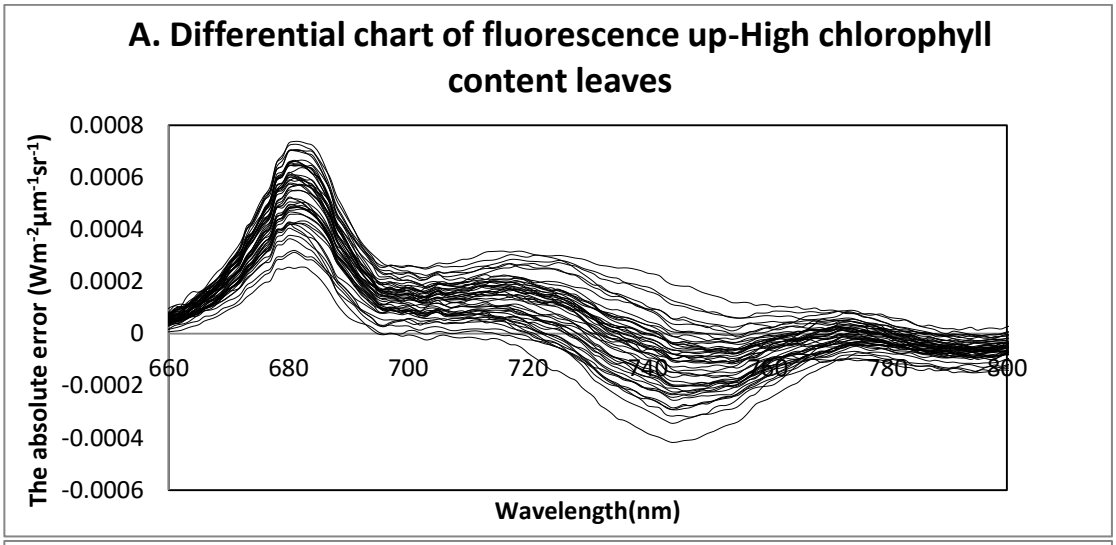
Table 5- 2 The relationship for six leaves after adding the FQE inversion code in simulation

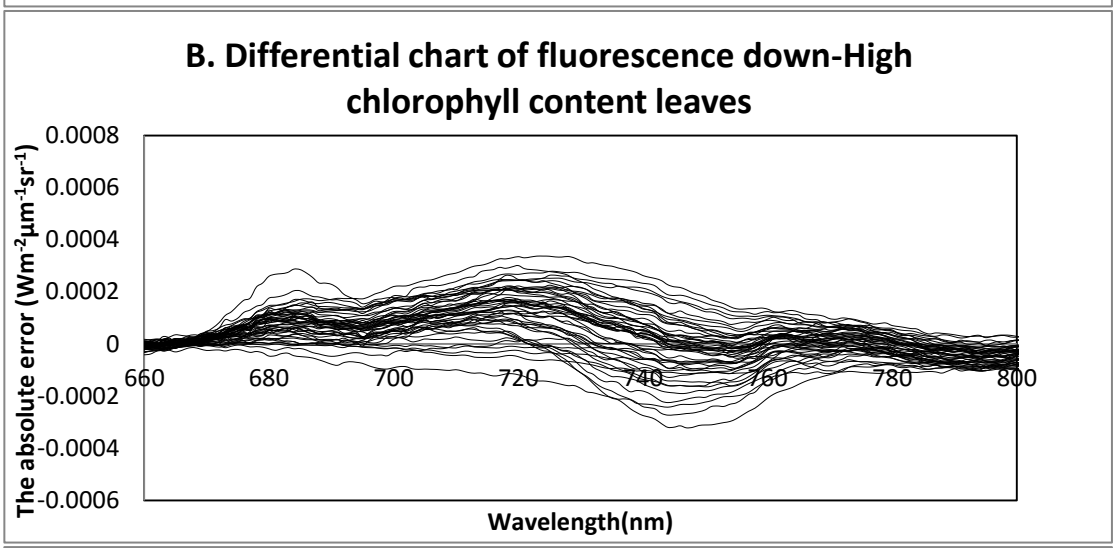
| INDEX | Fluorescence up for high chlorophyll content leaves | | | | | |
|---|---|---|---|---|---|---|
| PEARSON | 0.98 | 0.98 | 0.98 | 0.98 | 0.98 | 0.98 |
| $R^2$ | 0.97 | 0.96 | 0.96 | 0.97 | 0.97 | 0.97 |
| | Fluorescence down for high chlorophyll content leaves | | | | | |
| PEARSON | 0.99 | 0.98 | 0.98 | 0.99 | 0.99 | 0.99 |
| $R^2$ | 0.97 | 0.97 | 0.96 | 0.97 | 0.97 | 0.97 |
| | Fluorescence up for low chlorophyll content leaves | | | | | |
| PEARSON | 0.99 | 0.98 | 0.98 | 0.99 | 0.98 | 0.98 |
| $R^2$ | 0.98 | 0.96 | 0.96 | 0.97 | 0.96 | 0.97 |
| | Fluorescence down for low chlorophyll content leaves | | | | | |
| PEARSON | 0.97 | 0.97 | 0.97 | 0.99 | 0.98 | 0.99 |
| $R^2$ | 0.94 | 0.95 | 0.94 | 0.97 | 0.97 | 0.97 |

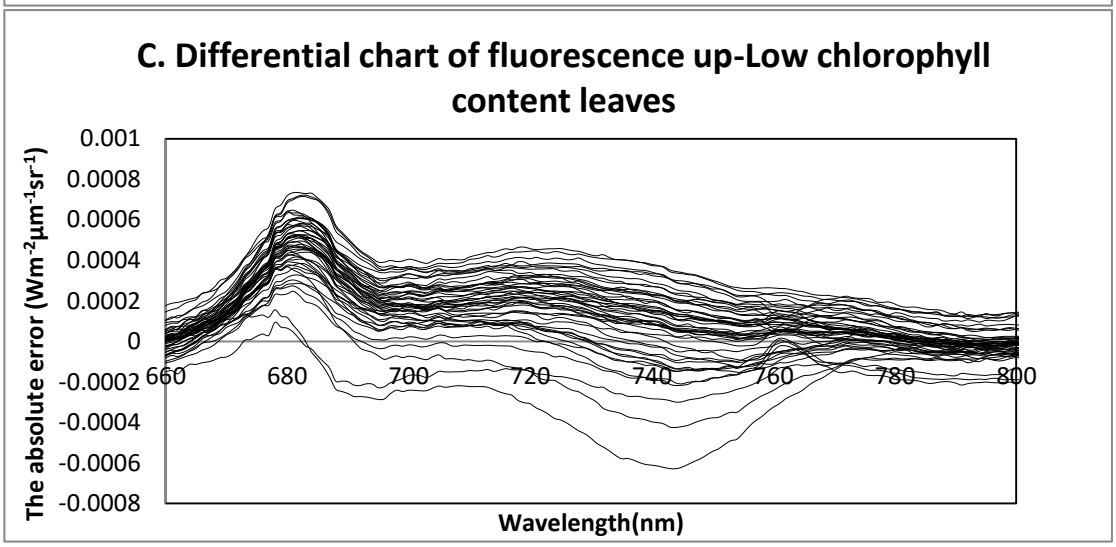
Table 5- 1 shows the relationship is good between the simulation fluorescence and the measured data. The R2 and PEARSON indexes are a little bit higher than the results which model has not been added the FQE inversion code. In total, the R2 is higher than 9.5. The model can simulate the fluorescence relatively good in whole wavelength. It can conclude that the relationship between the simulation data and measured data are good by just using R2 and PEARSON indexes.

### 5.3. The absolute and relative error charts

When using the simulated values to subtract the measured fluorescence, the absolute error between the simulation without FQE inversion and measured fluorescence can be calculated from 660nm to 800nm. For the high and low chlorophyll contents leaves, there are four subfigures in Figure 5- 2 for both upward and downward fluorescence. Every subfigure will show absolute error for one condition.


A. Differential chart of fluorescence up-High chlorophyll content leaves
The absolute error (Wm-2μm-1sr-1)
0.0008
0.0006
0.0004
0.0002
0
-0.0002
-0.0004
-0.0006
660
680
700
720
740
760
780
800
Wavelength(nm)


B. Differential chart of fluorescence down-High chlorophyll content leaves
The absolute error (Wm-2μm-1sr-1)
0.0008
0.0006
0.0004
0.0002
0
-0.0002
-0.0004
-0.0006
660
680
700
720
740
760
780
800
Wavelength(nm)


C. Differential chart of fluorescence up-Low chlorophyll content leaves
The absolute error (Wm-2μm-1sr-1)
0.001
0.0008
0.0006
0.0004
0.0002
0
-0.0002
-0.0004
-0.0006
-0.0008
660
680
700
720
740
760
780
800
Wavelength(nm)

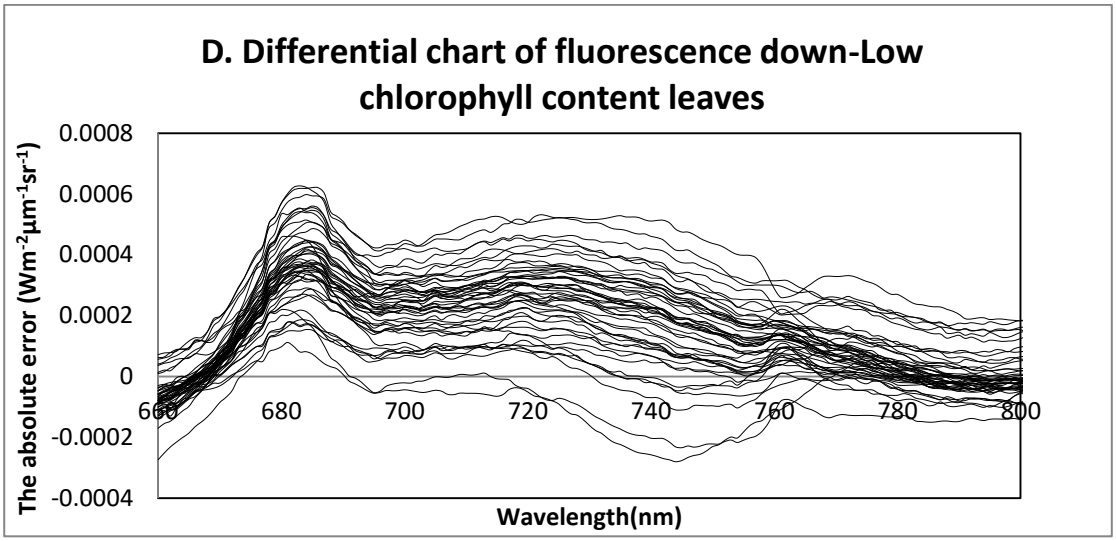
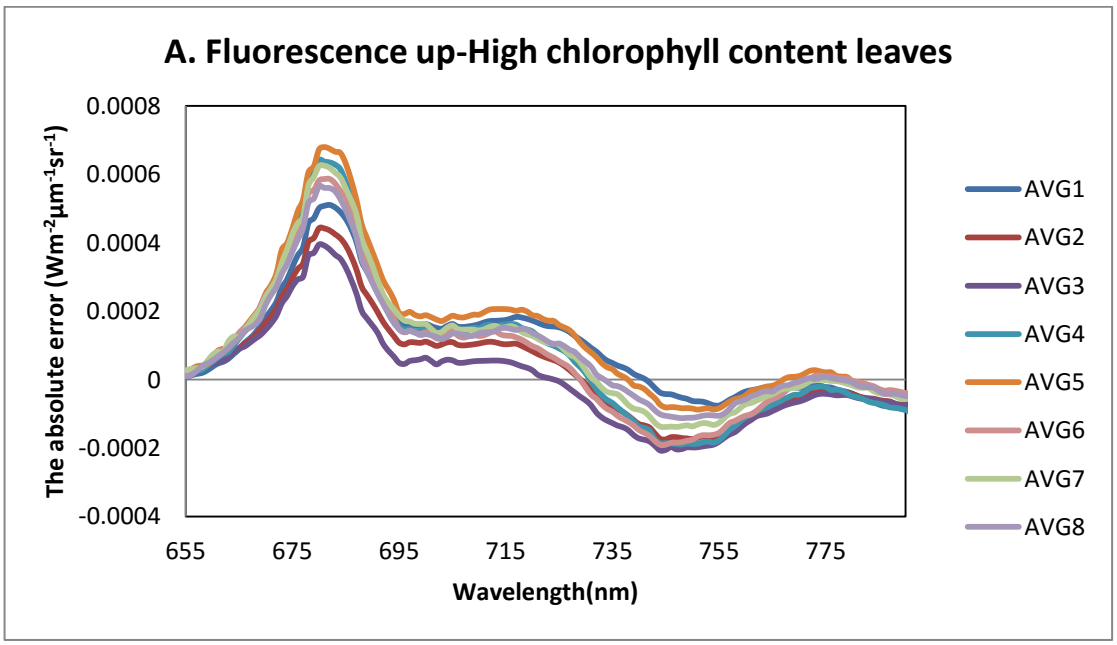
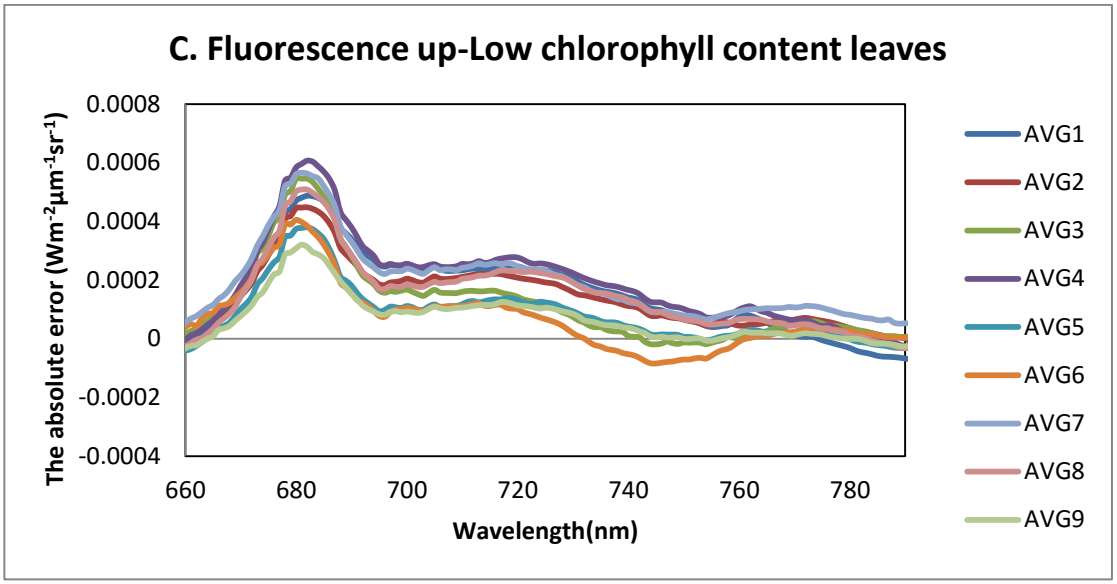
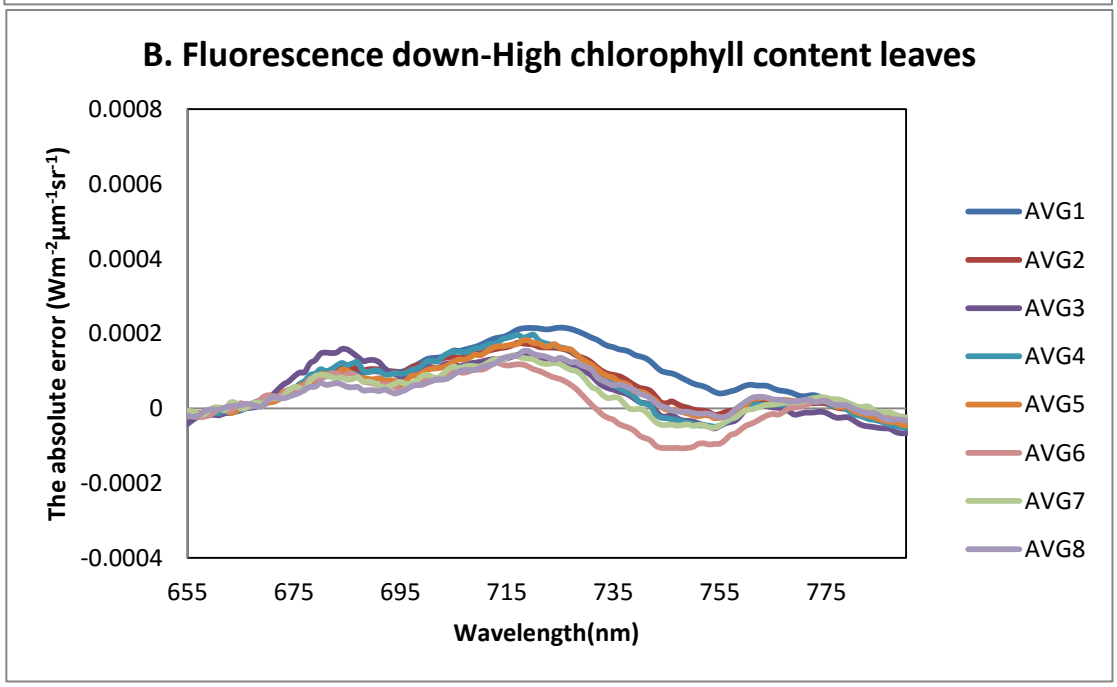



Figure 5- 2 The fluorescence absolute error between simulation and measurement for the all leaves

From the graphs of the model absolute error (measured minus simulation values), see Figure 5- 2, it appears that the simulated fluorescence is consistently higher than the measured fluorescence. For the fluorescence up, the simulated fluorescence is greater than the measured fluorescence especially around the 687nm in both high and low chlorophyll content leaves. For the fluorescence down, the fluorescence is overestimated around the 720nm in the high chlorophyll content leaves. Than the absolute error curve will decline after 720nm. In the low chlorophyll content leaves, the fluorescence is not only overestimated around the 720nm and also around the 687nm. The model always overestimates the fluorescence in the whole wavelength especially in the first peak.




C. Fluorescence up-Low chlorophyll content leaves
The absolute error (Wm-2µm-1sr-1)
0.0008
0.0006
0.0004
0.0002
0
-0.0002
-0.0004
660
680
700
720
740
760
780
Wavelength(nm)
AVG1
AVG2
AVG3
AVG4
AVG5
AVG6
AVG7
AVG8
AVG9


B. Fluorescence down-High chlorophyll content leaves
The absolute error (Wm-2µm-1sr-1)
0.0008
0.0006
0.0004
0.0002
0
-0.0002
-0.0004
655
675
695
715
735
755
775
Wavelength(nm)
AVG1
AVG2
AVG3
AVG4
AVG5
AVG6
AVG7
AVG8

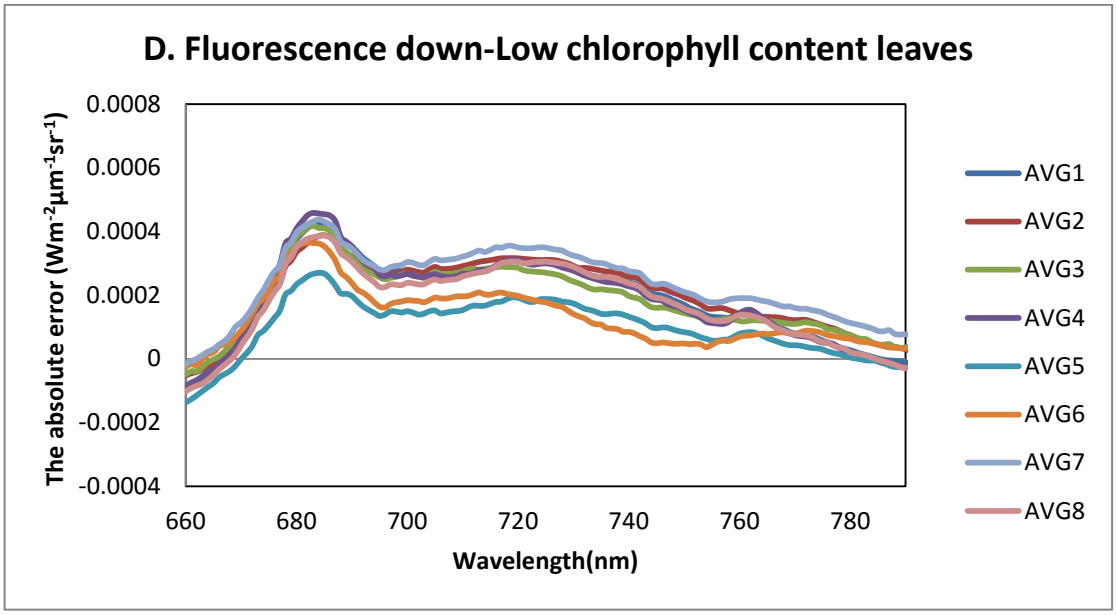
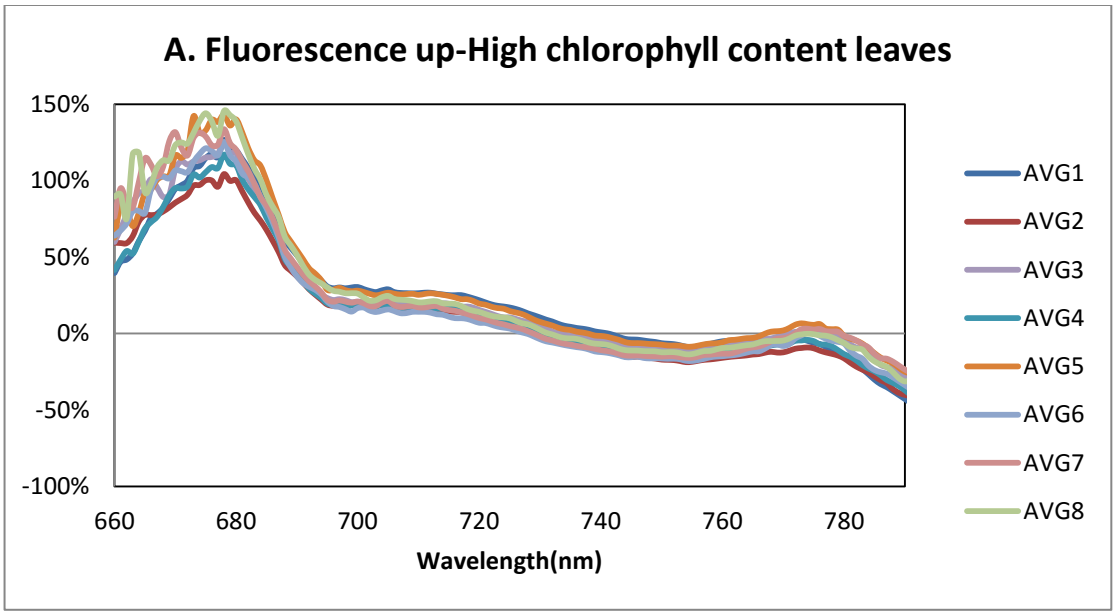
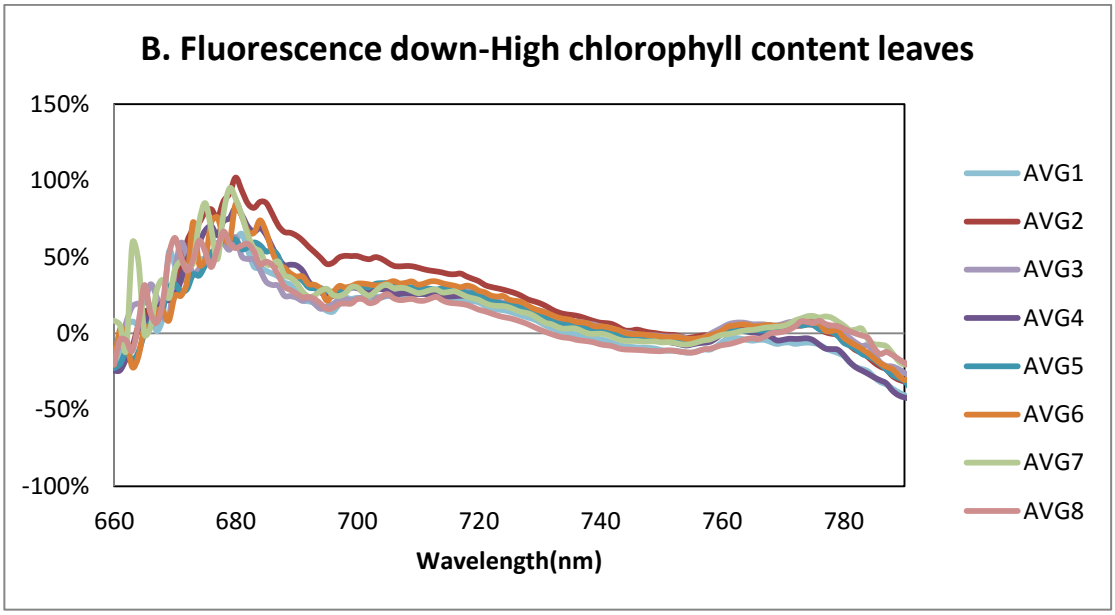
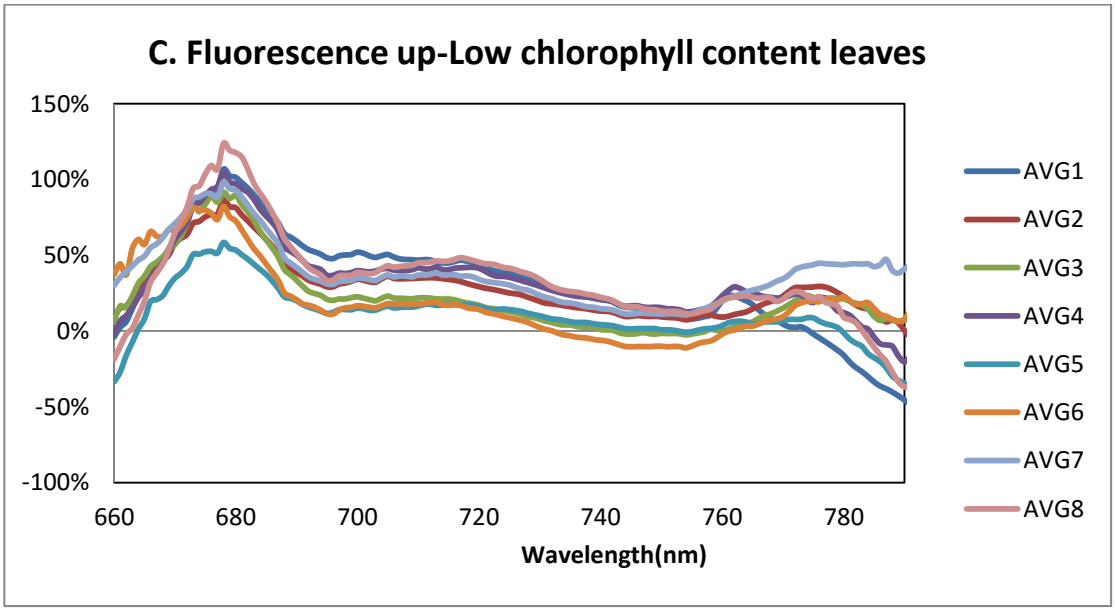



Figure 5- 3 The fluorescence absolute error between simulation and measurement for every measurement leaves

Because the measurement is in the one natural condition in every time, an average spectrum of the absolute error for all samples is provided as well (Figure 5- 3). For one group of measured data in the same natural condition, it is suitable to average the absolute value in one measurement condition.
In these figures, it is easy to see that the model overestimates the fluorescence around the 687nm except the high chlorophyll contents leaves in downward side. For the fluorescence up in high chlorophyll contents leaves, the first peak is overestimated, than the absolute error declines. In the second peak in 740nm, the absolute is negative and close to 0.For the fluorescence down, the curves in both up and down direction show the same tendency to overestimate the fluorescence in simulation. The model especially overestimated around the 670nm. Than the absolute error curve declines slowly. So the performance in first peak of fluorescence curves is not good.




B. Fluorescence down-High chlorophyll content leaves
150%
100%
50%
0%
-50%
-100%
660
680
700
720
740
760
780
Wavelength(nm)
AVG1
AVG2
AVG3
AVG4
AVG5
AVG6
AVG7
AVG8


C. Fluorescence up-Low chlorophyll content leaves
150%
100%
50%
0%
-50%
-100%
660
680
700
720
740
760
780
Wavelength(nm)
AVG1
AVG2
AVG3
AVG4
AVG5
AVG6
AVG7
AVG8

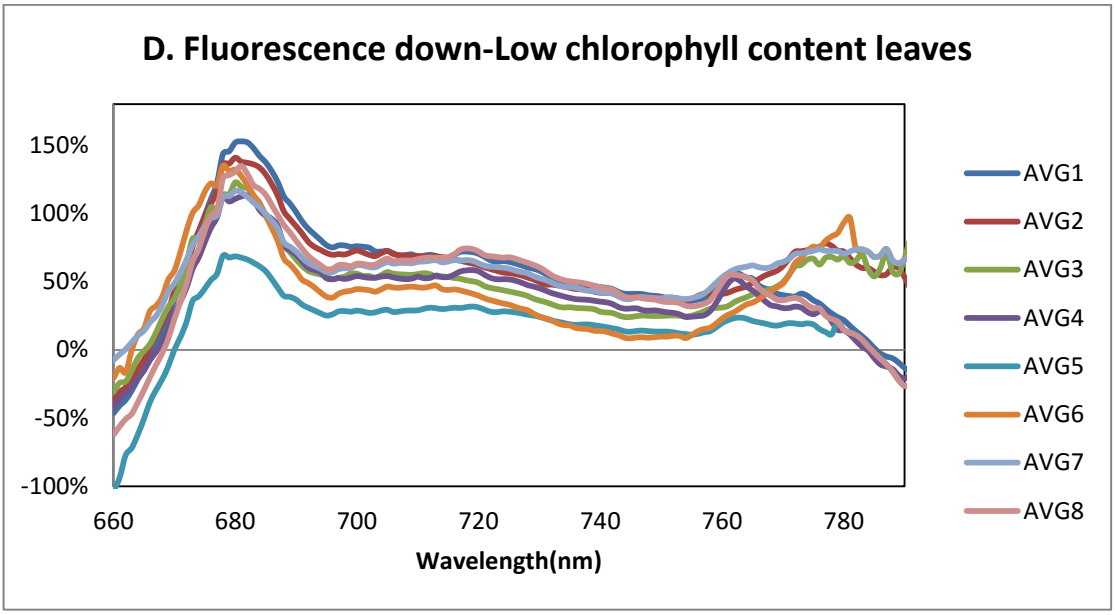



Figure 5- 4 The fluorescence relative error between simulation and measurement for every measurement

In the relative error charts, the simulated fluorescence is always overestimated around 687nm in each condition. So the first peak in the simulation fluorescence curve is higher than the actual condition. Because the relative error between the simulated and measured fluorescence is over than 100%, it can be concluded that the model has a bad performance in the first peak. And the other part of the differential percentage is less than 50% especially above the 700nm. In the second peak, the relative error is close to 0 in high chlorophyll content leaves. For the low content leaves, the first peaks in fluorescence curve are overestimated. The relative error is always higher than the relative error for high chlorophyll content leaves except the first peak. The model can simulate the fluorescence relative well above the 700nm.

### 5.4. Parameters comparison

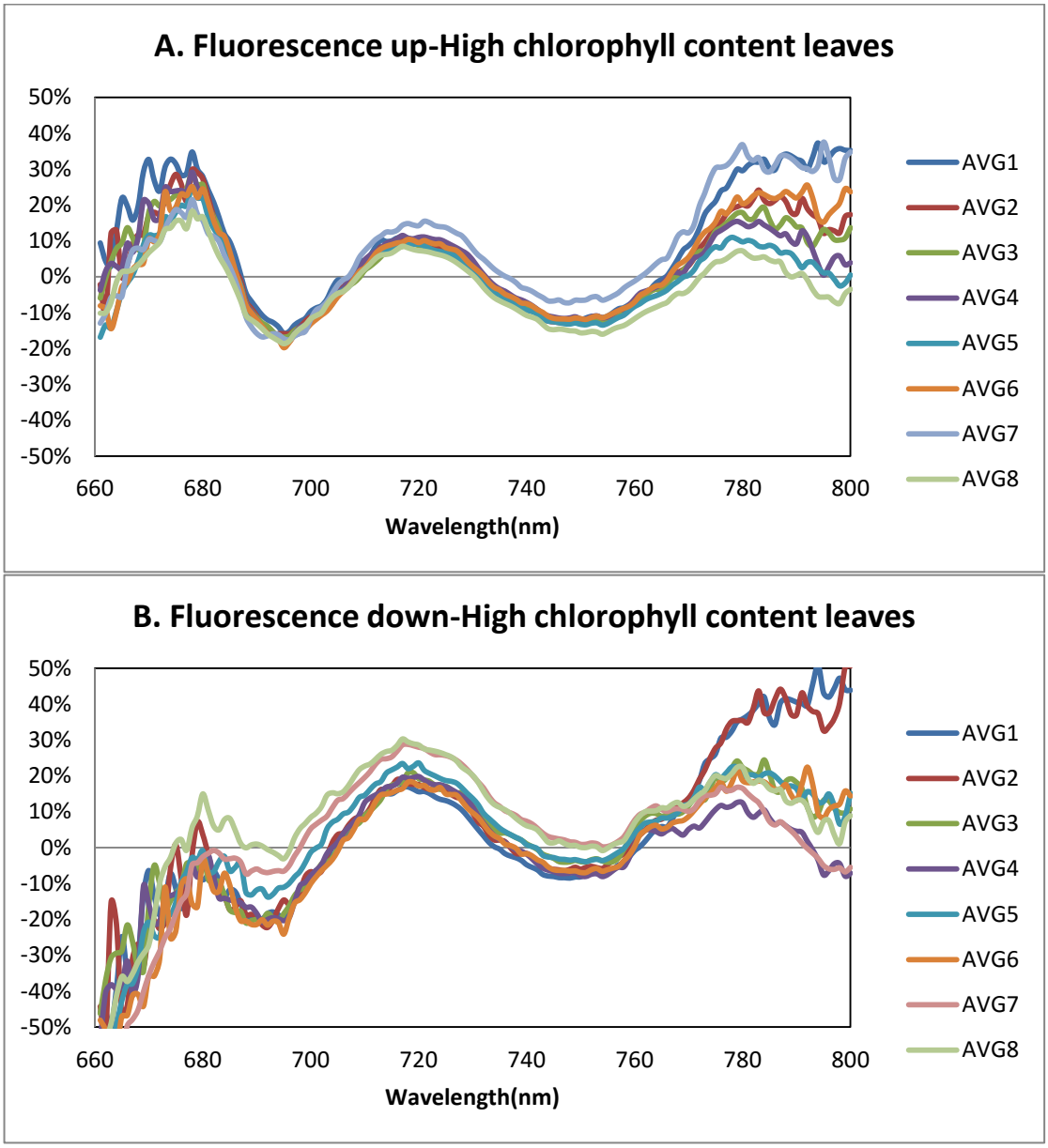
After model improvement, the FQE inversion code has been input in model. The model will retrieve all parameters which include FQE II and I. It is aimed to have a good fluorescence simulation.
Without the FQE inversion code, the FQE II and I were set as 0.01 and 0.002. In the complex weather condition, it is not fit for the all plants.
The Prospect parameters are almost identical when using the retrieval with or without FQE. The prospect parameters are retrieved by the reflectance and the transmittance data. The FQE does not affect reflectance and transmittance. The FQE is only affected by the measured fluorescence. The FQE values change a lot after retrieving the FQE value.

Table 5- 3 The inversion FQE value

| high chlorophyll content leaves | | | | |
|---|---|---|---|---|
| FQE1 (dimensionless) | 0.003 | 0.004 | 0.004 | 0.004 |
| FQE2 (dimensionless) | 0.006 | 0.006 | 0.006 | 0.005 |
| low chlorophyll content leaves | | | | |
| FQE1 (dimensionless) | 0.002 | 0.003 | 0.002 | 0.004 |
| FQE2 (dimensionless) | 0.006 | 0.006 | 0.005 | 0.010 |
| high chlorophyll content leaves on the top | | | | |
| FQE1 (dimensionless) | 0.004 | 0.003 | 0.004 | 0.004 |
| FQE2 (dimensionless) | 0.005 | 0.004 | 0.005 | 0.005 |
| high chlorophyll content leaves on the bottom | | | | |
| FQE1 (dimensionless) | 0.003 | 0.003 | 0.003 | 0.004 |
| FQE2 (dimensionless) | 0.004 | 0.005 | 0.005 | 0.005 |
| low chlorophyll content leaves on the top | | | | |
| FQE1 (dimensionless) | 0.002 | 0.002 | 0.003 | 0.002 |
| FQE2 (dimensionless) | 0.005 | 0.005 | 0.005 | 0.005 |
| low chlorophyll content leaves on the bottom | | | | |
| FQE1 (dimensionless) | 0.002 | 0.003 | 0.003 | 0.001 |
| FQE2 (dimensionless) | 0.006 | 0.007 | 0.006 | 0.005 |

The FQE II total decrease in all leaves. And the FQE I stay as 0.002 or increase a little. It has been known that the FQE II makes a decision on the first peak of the fluorescence. In the simulation without the FQE value, the simulations in the first peak are always overestimated. The FQE II is needed to decrease. Normally the second peak in simulation is fitted relatively well. So the FQEI only has little change. In Figure 5- 4, some values in relative error curve are negative. The FQE I is needed to increase a little to reproduce more fluorescence in 740nm. These inversion FQE value can reflect the weather condition as immolation and temperature condition and do more accurate simulation.

## 5.5. The relative error charts by simulating with the FQE inversion code model



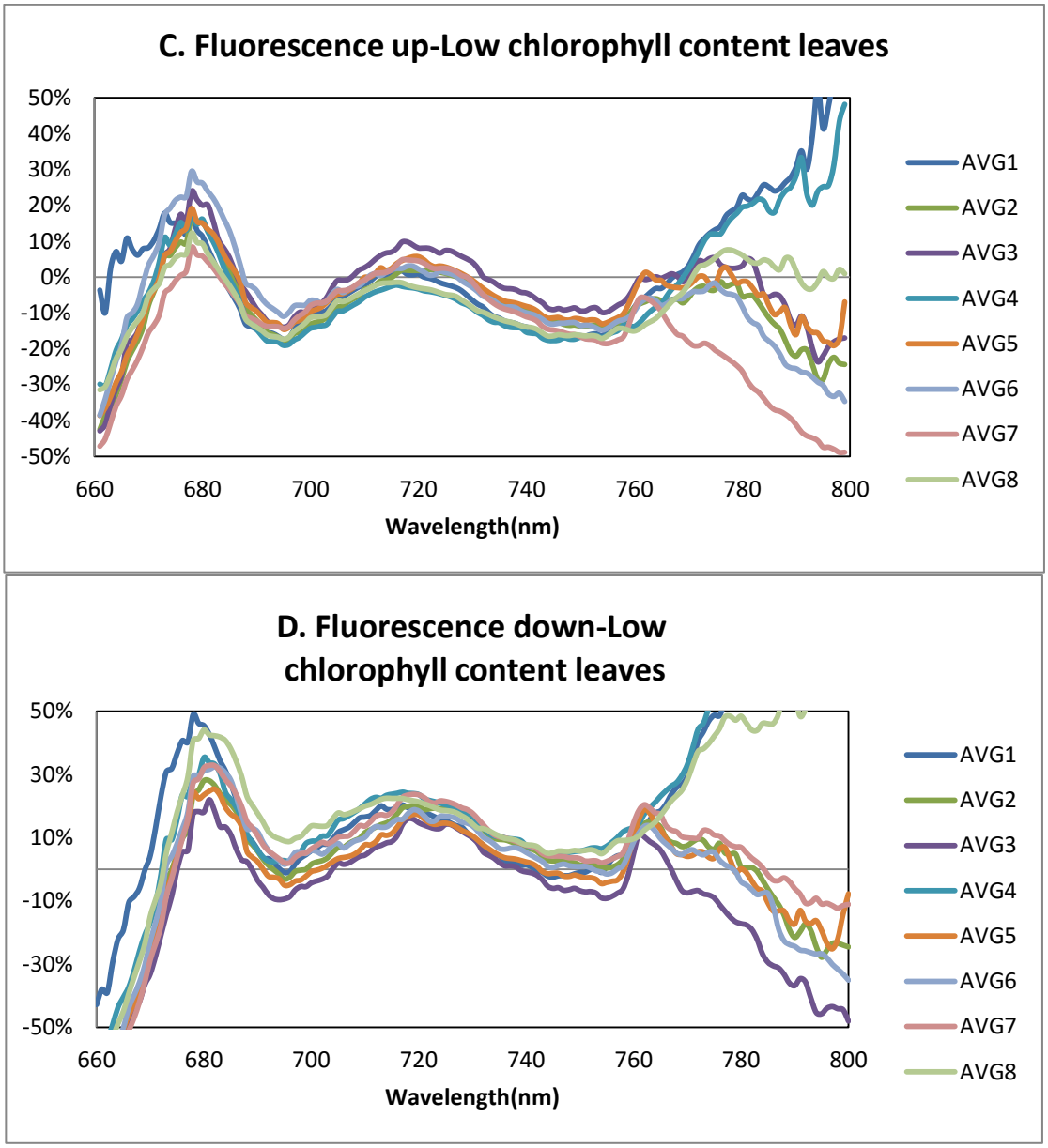



Figure 5- 5 The fluorescence relative error between simulation with FQE inversion code and measurement for every measurement

Both the absolute and the relative (normalized by measured fluorescence) error drops after tuning FQE. It can be concluded that the simulation results is better than the simulation fluorescence before. The differential value by using the simulation data mines the measured data is lower than before. The differential of the percentage decreases from 150% to 20% in the first peak in the fluorescence up curve. The second peak of fluorescence curve also reduces to the 20%. Comparing the results before, the simulation results is better than the simulation fluorescence by the model without the FQE inversion code.

### 5.6. The relative error on peak value

In the fluorescence study, the peak value is a very important index in fluorescence simulation. The peak value can reflect the photosynthesis condition in the plants. Also the peak values are related to the photosystem FQE II and I in the simulation. With better peak fitting, we can get more accurate simulation.

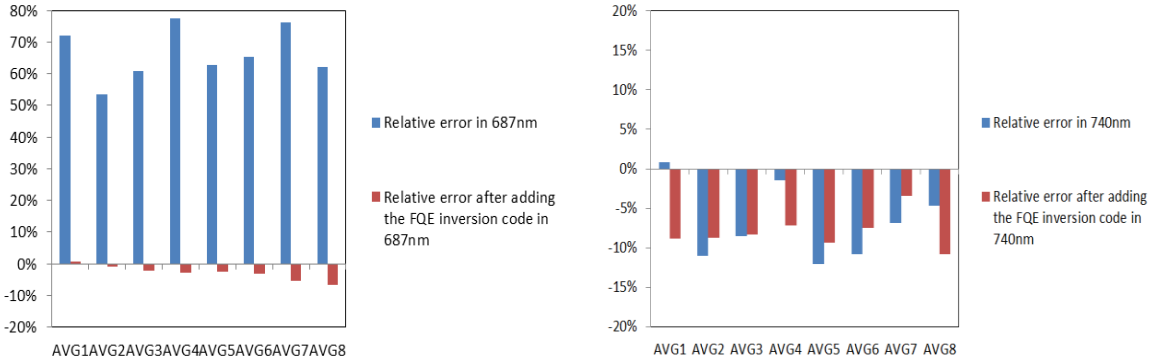



Figure 5- 6 The relative error on the upward for high chlorophyll content leaves

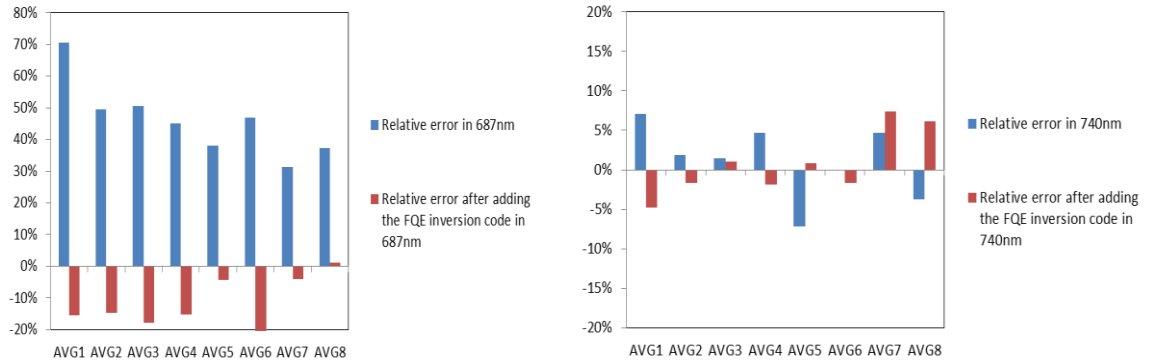



Figure 5- 7 The relative error on the downward for high chlorophyll content leaves

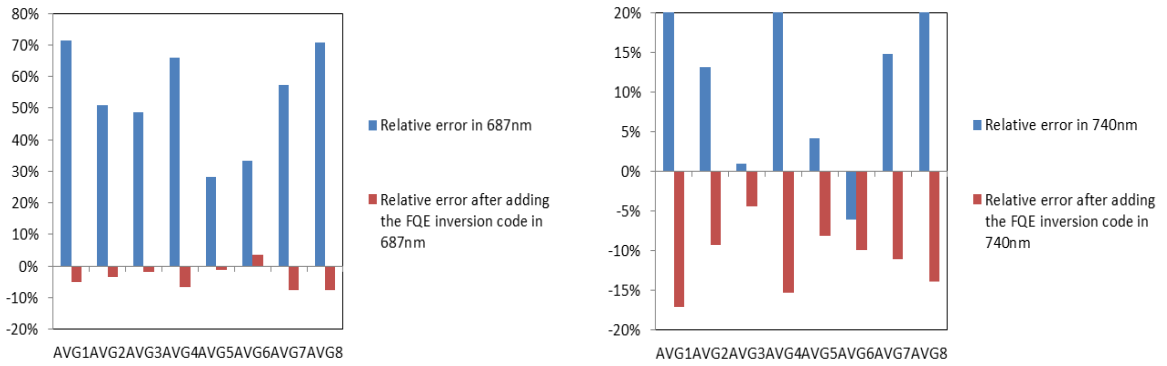



Figure 5- 8 The relative error on the upward for low chlorophyll content leaves

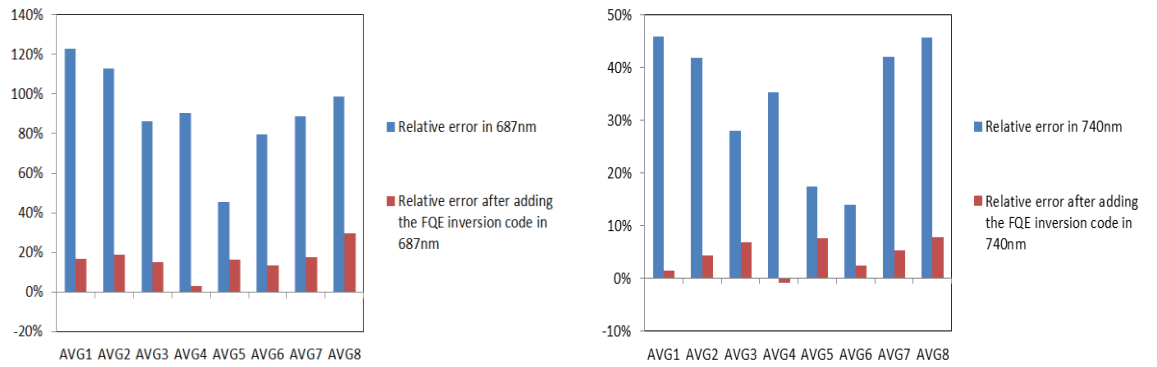



Figure 5- 9 The relative error on the downward for low chlorophyll content leaves

From Figure 5- 6 to Figure 5- 9, it is easy to see the relative errors of peak value have decreased a lot in the first peak for both high and low chlorophyll content leaves. Also the FQE II values have changed (see Table 5- 3). With the little change of FQE I , the improvement in the second is not notabl. But the rlative error still has a little decrease. The simulation without FQE inversion code is quite good. The relative error at the range from -20% to 20%. So the improvement is not notable. For the sechond peak relative error on the downward for low chlorophyll content leaves, it has a decrease to improve the simulation. So the FQE inversion code is successful to control the simulation result in an acceptable range. The relative errors almost are controlled between -20% and 20%.

# 6. CONCLUSION

The first simulation by the Fluspet model is not good to reproduce the fluorescence under the natural condition. The fluorescence is always overestimated in both upward and downward sides. The peak values of the simulation fluorescence are especially higher than the measured condition. So the Fluspect model is not satisfactory to do the simulation under the natural condition.

The main problem is that the RTM inversion model only retrieve six parameters of the plants. These retrieved parameters can show the chlorophyll contents, but the FQE parameters which depend on the status of the photosystem in the plants are set as a basic value. With the default value, the fluorescence spectrum can't be simulated very well. In the natural condition, the photosystems are not same in different solar light condition. The setting of the value will lead to overestimated or underestimated in the model simulation. In this study, the FQE II and I were set as 0.01 and 0.002. With these values, the results for the different chlorophyll contents leaves are higher than the measured data. These values are too big than the actual condition.

Tuning FQE to the measured fluorescence spectra showed that the original values of FQE II and I were too high. It is easy to see that these values which were retrieved by FQE inversion code decrease. By retrieving the FQE values, the model preforms well in simulation the fluorescence in the natural conditions.

The simulation results with the FQE inversion code are still improved. The differential percentages of the fluorescence up for the high chlorophyll content leaves between the simulation result and the measured data in the first peak reduce from over 150% to 20%. For the low content leaves, the differential percentage of the fluorescence up decrease 20%. For the second peak of fluorescence in both high and low chlorophyll content leaves, the percentage of difference reduced to 20% in both upward and downward side. The simulation results are acceptable after model improvement. The significance of the FQE was yet unknown. The big problem in measuring FQE is that the signal of photosystem II and I are mixed. The FQE values also depend on the weather condition. However, the retrieved values of the FQE are still needed to be tested and verified.

It can be concluded that the Fluspect model can simulate the Fluorescence under the natural condition. But the values of FQE need to be set very accurately. Depend on the weather condition and the feature of plants, the photosystem II and I will vary. After adding the FQE inversion code in the model, the RTM inversion model can give more accurate FQE value. With these values, the simulation results will quite good by comparing the measured fluorescence. We can actually simulate how FQE1 and FQE2 vary with illumination and temperature under the natural conditions, but still we need some realistic reference values to star with.

# LIST OF REFERENCES


Combal, B., Baret, F., & Weiss, M. (2002). Improving canopy variables estimation from remote sensing data by exploiting ancillary information. Case study on sugar beet canopies. *Agronomie*, *22*(2), 205–215.

Fromme, P., & Mathis, P. (2004). Unraveling the photosystem I reaction center: a history, or the sum of many efforts. *Photosynthesis Research*, *80*(1-3), 109–24.

Hall, F. G., Huemmrich, K. F., Goetz, S. J., Sellers, P. J., & Nickeson, J. E. (1992). Satellite remote sensing of surface energy balance: Success, failures, and unresolved issues in FIFE. *Journal of Geophysical Research*, *97*(D17), 19061.

Lichtenthaler, H. K., Buschmann, C., Rinderle, U., & Schmuck, G. (1986). Application of chlorophyll fluorescence in ecophysiology. *Radiation and Environmental Biophysics*, *25*(4), 297–308.

Rosema, A., & Zahn, H. (1997). Laser pulse energy requirements for remote sensing of chlorophyll fluorescence. *Remote Sensing of Environment*, *62*(1), 101–108.

Sun, L., Zhou, S., Yan, J., Jiang, D., & Li, J. (2003). Application of laser induced fluorescence technique on detection of pesticide leftover. *Laser & Infrared*. Retrieved from https://scholar.google.com/scholar?hl=zh-CN&q=Application+of+laser+induced+fluorescence+technique+on+detection+of+pesticide+leftover.&btnG=&lr=#0

Tang, S., Chen, J. M., Zhu, Q., Li, X., Chen, M., Sun, R., … Xie, D. (2007). LAI inversion algorithm based on directional reflectance kernels. *Journal of Environmental Management*, *85*(3), 638–48.

van der Tol, C., Verhoef, W., Timmermans, J., Verhoef, a., & Su, Z. (2009). An integrated model of soil-canopy spectral radiances, photosynthesis, fluorescence, temperature and energy balance. *Biogeosciences*, *6*(12), 3109–3129.

Van Wittenberghe, S., Alonso, L., Verrelst, J., Moreno, J., & Samson, R. (2015). Bidirectional sun-induced chlorophyll fluorescence emission is influenced by leaf structure and light scattering properties — A bottom-up approach. *Remote Sensing of Environment*, *158*, 169–179.

Verhoef, a., & Allen, S. J. (2000). A SVAT scheme describing energy and CO2 fluxes for multi-component vegetation: Calibration and test for a Sahelian savannah. *Ecological Modelling*, *127*(2-3), 245–267.

Weiss, M., Baret, F., Myneni, R. B., Pragnre, A., & Knyazikhin, Y. (2000). Investigation of a model inversion technique to estimate canopy biophysical variables from spectral and directional reflectance data. *Agronomie*, *20*(1), 3–22.

Wittenberghe, S. Van, Alonso, L., Verrelst, J., & Hermans, I. (2014). BIOHYPE : What can we learn from hyperspectral solar-induced chlorophyll fluorescence of urban tree leaves , (November).