Modeling of the radiometric characteristics of a simulated fluorescent imager

Alexander Granica

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M.S. Degree Thesis

The M.S. Degree Thesis of Alexander J. Granica has been examined and approved by the thesis committee as satisfactory for the thesis requirement for the Master of Science degree.

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Date 6/7/96
Modeling of the Radiometric Characteristics of a Simulated Fluorescent Imager

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Modeling of the Radiometric Characteristics of a Simulated Fluorescent Imager

by

Alexander J. Granica

Submitted to the
Chester F. Carlson Center for Imaging Science
in partial fulfillment of the requirements
for the Master of Science Degree
at the Rochester Institute of Technology

Abstract

The purpose of this study was to determine whether changes in stimulated plant fluorescence could be detected by a remote sensing system. By measuring the changes in fluorescent signal the health status of vegetation can be determined. The plant condition can be assessed by viewing the ratio 690 nm/730 nm from the fluorescent spectra at the leaf level. The question is whether the change in fluorescent spectra (change in the ratio) can be seen at a remote location. In order to address this question a theoretical scenario was developed and the expected signal at a remote location was determined through radiometric calculations. The scenario involved determining the signal, at three different standoff distances, from a soybean plant under six different plant conditions.

The fluorescent signal of a plant at the leaf level was taken (from previous research) and the effects of the canopy factored into the signal. This was accomplished through use of a theoretical canopy model. Lowtran was used to model the atmospheric effects. The atmospheric and sensor system effects were incorporated through a series of radiometric calculations. This provided the fluorescent ratio (690 nm/730 nm) for three different sensor standoff distances and six different plant conditions.

It was found that the six different soybean conditions could be distinguished from one another when measured at a standoff of 10 or 30 meters. In order for all six
conditions to be distinguished, the canopy density of the plant had to have a value above 1 and 1.5 in the 10m and 30 m cases. Also the measured signal had to be averaged over four detector resolution elements. In the case of a 300 meter standoff the different plant conditions could be seen if the plant canopy had a leaf area index (canopy density) of at least 2.3 and the signal was averaged over 16 resolution elements. The collection conditions, leaf area index, canopy structure, soil reflectivity, atmospheric conditions, and sensor system response were all found to affect the fluorescent ratio measured.
Acknowledgments

I would like to thank my thesis committee: Dr. John Schott, Dr. Jonathan Arney, and Dr. William Philpot for their personal commitment to this research. Their time and effort are the reasons behind the success of this project and their support is very appreciated.

I would like to especially thank my advisor Dr. John Schott. His advice, expertise, and guidance not only made this research possible but also made it enjoyable.

The financial and technical support of DOE / Special Technologies Laboratory (Santa Barbara Operations) was instrumental in the development and completion of this project. Their support and interest in this research is very appreciated.

Personal thanks to John DiBenedetto and Steve Lutz for exposing me to the fine art of Laser Induced Fluorescence and their patience with my questions.

A special thanks to Dr. Daniel Schuresko for his encouragement and support in my abilities as an Imaging Scientist.
Dedication

I would like to dedicate this paper to all my family and friends. They helped me complete this research by reminding me there is more to life than Laser Induced Fluorescence. Without their companionship and love this paper would not have been possible.

Thanks Mom and Dad!

Hey Everybody, Guess What?
I’m Done.
Table of Contents

1. INTRODUCTION

2. BACKGROUND

2.1 PLANT FLUORESCENCE
2.2 KAUTSKY EFFECT (INDUCTION KINETICS)
2.3 SPECTRAL FLUORESCENCE
  2.3.1 Excitation and Collection Wavelengths
  2.3.2 Effects of Chlorophyll Concentration
  2.3.3 Leaf Side (Upper versus Lower)
  2.3.4 Sun/Shade Leaves
  2.3.5 Species Variation (Monocots/Dicots)
2.4 PLANT STRESS
  2.4.1 Herbicide Stress
  2.4.2 Daylight Cycle (Short Term Days)
  2.4.3 Water Stress
  2.4.4 Temperature
  2.4.5 Mechanical Injury
  2.4.6 Nitrogen
  2.4.7 More Nutrients
  2.4.8 Senescence
  2.4.9 Soil Salinity
  2.4.10 High Ambient Light Levels
  2.4.11 UV-B Radiation
  2.4.12 Air Pollution
  2.4.13 Acid Rain
  2.4.14 Metals
  2.4.15 Summary
2.5 PARAMETERS FOR COLLECTING PLANT FLUORESCENCE
  2.5.1 Excitation Intensity
  2.5.2 Excitation Wavelength
  2.5.3 Ambient Light
  2.5.4 Modeling of Canopy Effects
  2.5.5 Canopy Model by Rosema, A.
  2.5.6 Canopy Model by Olioso, A.
2.6 THESIS OBJECTIVES

3. PROCEDURE FOR THE CALCULATION OF CANOPY LEVEL FLUORESCENT EFFICIENCY

3.1 ATTENUATION OF RADIATION DUE TO THE CANOPY
3.2 EXCITING RADIATION REACHING A CANOPY DEPTH
3.3 LEAF FLUORESCENCE DUE TO EXCITING RADIATION
3.4 FLUORESCENT SIGNAL REACHING THE TOP OF THE PLANT CANOPY
3.5 DETERMINE THE FLUORESCENCE REACHING THE CANOPY TOP FROM A PARTICULAR CANOPY LAYER
3.6 AMOUNT OF SIGNAL EXITING THE CANOPY FROM FLUORESCENCE REFLECTING FROM THE SOIL
3.7 AMOUNT OF FLUORESCENT SIGNAL EXITING THE CANOPY PRODUCED FROM EXCITING RADIATION REFLECTING OFF THE SOIL

viii
3.8 Overall Fluorescent Signal Leaving the Canopy
3.9 Canopy Depth Affects on the Fluorescent Efficiency at Bands 690nm and 730nm
3.10 Adjustment of Process to Fit Procedure Used in the Final Calculations

4. Procedure for the Radiometric Calculation of Remotely Detected Laser Induced Fluorescence of Vegetation

4.1 Divergence of the Laser Illumination
4.2 Radiant Intensity of the Laser Source
4.3 Irradiance of the Laser Source onto the Target
4.4 Number of Photons Irradiated onto the Target
4.5 Number of Fluorescent Photons Emitted from the Plant
4.6 Radiant Excitance of Fluorescent Energy from the Plant
4.7 Radiance of Fluorescent Energy from the Plant
4.8 Amount of Reflected and Upwelled Radiance
4.9 Amount of Radiance Reaching the Sensor
4.10 F# of Detection System
4.11 Determine G# of the Detection System
4.12 Irradiance on the Detector
4.13 Radiant Flux on the Detector
4.14 Conversion of Radiant Flux from Watts to Photons
4.15 Signal Output from the Detector
4.16 Signal to Noise Ratio of the Detector
4.17 Noise (Standard Deviation) in the Camera Signal
4.18 Radiometric Signals from the Wavebands 690 nm and 730 nm
4.19 Ratio of the Two Wavebands 690 nm and 730 nm
4.20 Standard Deviation in the Index Value
4.21 Average Index Signal
4.22 Standard Deviation in the Average Index
4.23 Average of the Index Noise
4.24 Overall Noise in the Index
4.25 Index and Standard Deviation for Both a Healthy and Stressed Plant
4.26 Separation of the Indexes over the LAI
4.27 Minimum Difference Needed to Distinguish a Change in Index
4.28 Determine the Separability of Different Plant Conditions

5. Modeled Scenario

5.1 Assumptions and Approach of Building the Scenario
5.1.1 Time of Day (Ambient Light)
5.1.2 Collection Point of View (Sensor Position)
5.1.3 Excitation Wavelength
5.1.4 Plant Type
5.1.5 Stress Conditions
5.1.6 Leaf Level Fluorescent Efficiency Values
5.1.7 Canopy Density
5.1.8 Canopy Fluorescent Efficiency
5.1.9 Prospect modeling of Plant Reflectivity
5.1.10 Lowran Modeling of the Atmosphere
5.1.11 Sensor Systems and Collection Scenarios
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1.12</td>
<td>Radiometric Calculations</td>
<td>138</td>
</tr>
<tr>
<td>6.</td>
<td>RESULTS</td>
<td>151</td>
</tr>
<tr>
<td>7.</td>
<td>CONCLUSIONS</td>
<td>185</td>
</tr>
<tr>
<td>8.</td>
<td>REFERENCES</td>
<td>193</td>
</tr>
<tr>
<td>9.</td>
<td>APPENDIX</td>
<td>206</td>
</tr>
<tr>
<td>9.1</td>
<td>CONTROL7 INPUT DATA</td>
<td>206</td>
</tr>
<tr>
<td>9.2</td>
<td>LOWTRAN CARD DECK FOR 10 METERS</td>
<td>211</td>
</tr>
<tr>
<td>9.3</td>
<td>LOWTRAN CARD DECK FOR 30 METERS</td>
<td>213</td>
</tr>
<tr>
<td>9.4</td>
<td>LOWTRAN CARD DECK FOR 300 METERS</td>
<td>215</td>
</tr>
</tbody>
</table>
List of Figures

FIGURE 1: ABSORPTION AND CHLOROPHYLL FLUORESCENCE SPECTRA ........................................... 20
FIGURE 2: LASER-INDUCED CHLOROPHYLL-FLUORESCENCE INDUCTION KINETICS ........................ 22
FIGURE 3: FLUORESCENCE EMISSION SPECTRA OF YOUNGER AND OLDER SPRUCE NEEDLES OF DIFFERENT CHLOROPHYLL CONTENT .......................................................................................... 26
FIGURE 4: FLUORESCENCE FROM THE UPPER/LOWER LEAF SIDE OF SUN AND SHADE LEAVES .......... 28
FIGURE 5: FLUORESCENCE OF THE UPPER/LOWER LEAF SIDES OF UNTREATED AND DCMU TREATED RADISH SEEDLINGS ............................................................................................................. 32
FIGURE 6: FLUORESCENCE OF A BEECH LEAF AT DIFFERENT EXCITATION WAVELENGTHS ............ 46
FIGURE 7: FLUORESCENCE BANDS AND INTENSITY AS A FUNCTION OF EXCITATION WAVELENGTH ...... 47
FIGURE 8: RED FLUORESCENCE SPECTRA IN DIFFERENT SUNLIGHT CONDITIONS ............................ 48
FIGURE 9: DAILY BEHAVIOR OF THE RFR INDEX (685/730 NM) AND OF THE PPFD ................................ 49
FIGURE 10: CHANGES OF THE RELATIVE CONTRIBUTION OF THE F690 AND F730 BANDS TO TOTAL FLUORESCENCE (OVER VARYING PPFD) ........................................................................................................ 50
FIGURE 11: FLUORESCENCE MEASURED AT 680 NM AND 730 NM OVER A 24 HOUR PERIOD ............... 51
FIGURE 12: DAILY CYCLE OF THE FLUORESCENCE SIGNALS AT 440 NM, 685 NM, AND 730 NM ........ 52
FIGURE 13: DAILY CYCLE OF THE FLUORESCENCE RATIOS F685/F730 AND F440/F685 .................. 53
FIGURE 14: FLUORESCENT INTENSITY VERSUS CANOPY DEPTH ................................................... 56
FIGURE 15: SIMULATIONS OF CANOPY FLUORESCENCE .................................................................... 57
FIGURE 16: SIMULATION OF Fcpf0/F_c730 FOR A SPHERICAL CANOPY AND DIFFERENT LEAF CHLOROPHYLL CONTENT .................................................................................................................... 58
FIGURE 17: SIMULATION OF Fcpf0/F_c730 RATIO FROM A STRESSED AND UNSTRESSED SOYBEAN ........ 59
FIGURE 18: THREE SOURCES OF A CANOPY FLUORESCENCE SIGNAL ................................................ 70
FIGURE 19: FLUORESCENT INTENSITY VERSUS CANOPY DEPTH ..................................................... 75
FIGURE 20: DIAGRAM OF THE FACTORS INFLUENCING LASER IRRADIANCE ......................................... 80
Figure 43: Signal to Noise Ratio for a Healthy and DCMU Treated Soybean Plant at a 10 Meter Standoff

Figure 44: Signal to Noise Ratio for a Healthy and DCMU Treated Soybean Plant at a 30 Meter Standoff

Figure 45: Signal to Noise Ratio for a Healthy and DCMU Treated Soybean Plant at a 300 Meter Standoff

Figure 46: Index and Range of Soybean at 300 Meters Without Pixel Averaging

Figure 47: Index and Range for a Healthy and DCMU Treated Soybean Determined at 10 Meters with a Background Soil with a Small Reflectivity Range

Figure 48: Minimum and Mean Differences Between Indexes from a Healthy and DCMU Treated Soybean Plant Using a 10 Meter Standoff (Small Soil Reflectivity Range)

Figure 49: Index and Range for a Healthy and DCMU Treated Soybean Determined at 10 Meters with a Background Soil with a Medium Reflectivity Range

Figure 50: Minimum and Mean Differences Between Indexes from a Healthy and DCMU Treated Soybean Plant Using a 10 Meter Standoff (Medium Soil Reflectivity Range)

Figure 51: Standard Deviation from the Index of a DCMU Treated Soybean Plant

Figure 52: Index and Ranges for Different Soybean Chlorophyll Levels and Two Different Soil Reflectivity Ranges Determined for a 10 Meter Standoff

Figure 53: Minimum and Actual Differences between Indexes for Different Chlorophyll Levels of Soybean Determined for a 10 Meter Standoff

Figure 54: Index and Range for a Healthy and DCMU Treated Soybean Determined at 30 Meters with a Background Soil with a Small Reflectivity Range

Figure 55: Minimum and Mean Differences Between Indexes from a Healthy and DCMU Treated Soybean Plant Using a 30 Meter Standoff (Small Soil Reflectivity Range)

Figure 56: Index and Range for a Healthy and DCMU Treated Soybean Determined at 30 Meters with a Background Soil with a Medium Reflectivity Range

Figure 57: Minimum and Mean Differences Between Indexes from a Healthy and DCMU Treated Soybean Plant Using a 30 Meter Standoff (Medium Soil Reflectivity Range)

Figure 58: Index and Ranges for Different Soybean Chlorophyll Levels and Two Different Soil Reflectivity Ranges Determined for a 30 Meter Standoff

Figure 59: Minimum and Actual Differences between Indexes for Different Chlorophyll Levels of Soybean Determined for a 30 Meter Standoff
List of Tables

Table 1: Differences in the Fluorescence Ratio F690/F730 Between the Upper and Lower Leaf Sides of Different Plants .......................................................... 29

Table 2: Differences in the Chlorophyll-Fluorescence Ratio F690/F735 in Leaves of Different Plants After DCMU Treatment and in Needles of Healthy (Damage Class 0) and Damaged Conifer Trees (Damage Class 2 or 3) .................................................. 34

Table 3: Factors Affecting Fluorescent Intensity and the Ratio 690/730 nm .................. 60

Table 4: Attenuation ($k_e$ and $k_r$) and Interception (K) Coefficients for Different Canopy Structures at a Leaf Chlorophyll Content of 40 $\mu$g/cm$^2$ .................................................... 67

Table 5: Attenuation ($k_e$ and $k_r$) Coefficients for a Spherical Canopy with Different Leaf Chlorophyll Content ................................................................. 68

Table 6: Ratio of Leaf Level Fluorescence (690 nm/730 nm) for a Soybean Plant ........ 113

Table 7: Fluorescent Efficiencies for Bands 690, 730 nm and Variables “a” and “b” ...... 122

Table 8: Input Parameters Used to Generate Four Canopy Fluorescent Efficiencies ......... 123

Table 9: Input Parameters Used to Generate Reflectance Curves in PROSPECT .......... 127

Table 10: Radiometric Input Parameters for Three Sensor Platforms ......................... 135

Table 11: Irradiance of the Laser on the Plant for 3 Sensor Systems ......................... 139

Table 12: Number of Resolution Elements Averaged at Each Sensor Platform ............ 161
1. **Introduction**

   As the concerns over our environment grow so does the desire to assess its condition quickly and accurately. This has caused much development in the area of remote sensing. One particular environmental interest is vegetation condition. Vegetation is an important part of our ecosystem. A significant change in the vegetation of an area has a major impact on many lifeforms in that area. By monitoring the changes in plants one can anticipate future problems with plant condition. In some cases, early detection of harmful conditions may allow intervention to prevent the problem from coming to fruition. Thus there is a need for a system that will accurately detect changes in plant condition before permanent damage is done. The capability of making this assessment over wide areas is also needed.

   In past research many people have described the effects different variables have on plant fluorescence. In current work, there is an attempt being made to measure these signatures remotely. Still it is widely unknown how comparable these remote fluorescent signals will be to actual plant condition. The thrust of this project was to estimate, based on published data, the potential utility of a remotely based system that stimulates and detects fluorescence of vegetation in order to measure the vegetation condition. This was done by utilizing the following: previous research to provide expected leaf level fluorescent signatures, a mathematical model to approximate the effects of a canopy on the signature, and radiometric calculations to determine the signal loss due to remote collection.

   Recent research has taken into account the effects of the canopy on the fluorescent signal. This paper goes beyond this by calculating what these signatures should be under actual remote sensing conditions.

   It has been shown at the leaf level that a plant under stress will have a different fluorescent signature than that of an unstressed plant [Lichtenthaler et. al, 1988]. Many
studies, using different experimental conditions, show this correlation. What is still undetermined is under what conditions, if any, weakening plant health can be remotely detected.

The first phase of this project was to determine, from past research, what fluorescent signals can be expected at the leaf level. The signals for a healthy and stressed plant were located. This information was taken from experiments that can be recreated at a remote distance. This involved deciding on which fluorescent measurement technique to use (Kautsky effect vs. spectral fluorescence, excitation wavelength, and collection wavelength).

It is known that many factors (plant species, type of stress, collection parameters, etc.) effect the fluorescent signal of vegetation at the leaf level. These factors make it hard to distinguish between healthy and stressed plants. Therefore the influence these factors have on leaf fluorescence was reviewed.

Past research has brought to light many of these variables influencing leaf fluorescence. However, little work has been done in trying to assimilate what all these issues do to the overall fluorescence at the plant level. The objective of the literature review was to determine all the issues that effect plant fluorescence. The major factors influencing leaf fluorescence were identified. Then, when it is possible, the variables influence on the signal was reduced (by choosing a situation that reduces influencing factors, example: measuring only one plant species instead of many). In creating this situation (or scenario) where fluorescence is to be measured, the conditions of the set up are specified. This in effect builds a theoretical experiment in order to see if it may be possible to measure such a signal in the real world. The factors whose influence could not be reduced were included in determining the expected fluorescent signature. The result of this background review was a known fluorescent signal, at the leaf level, for a healthy and stressed plant in a given situation (or scenario).
The developed scenario involved calculating the fluorescent signal from a soybean plant under six different conditions. The conditions being a soybean plant before and after a herbicide stress; and the plant at four different chlorophyll concentration levels. The fluorescent signal from these conditions was determined for three different sensor systems. Each system was designed to be utilized at a different standoff distance. The standoff distances related to a greenhouse collection (10 meters), a field based collection (30 meters), and an air based collection (300 meters). The sensor systems were modeled using a nadir point of view and a 5 PM collection time.

Once the vegetation fluorescence, for a specific situation, was known at the leaf level the next step was to consider the problems involved in remote detection. The main factors are canopy, ambient light, atmospheric and radiometric effects.

Changing daylight conditions and canopy interactions are concerns that have not been major problems in the laboratory setting. Ambient light influences the amount of photosynthetic activity a plant undergoes. Therefore it can alter the fluorescent signal. The change in the fluorescent signal due to ambient light was found in the literature. It's effects were taken into account when creating the theoretical situation to be modeled. This was done by choosing a specific collection time (5 PM). The outcome from these steps was the leaf level fluorescent signatures of healthy and stressed plants in a theoretical field situation.

In order to make a distant fluorescent measurement, the instrument needs to look at the whole plant canopy instead of just one leaf. However, the canopy itself can have many effects on the signal of interest. The effects on the signal were determined with the use of a canopy model. In past research several mathematical models have been devised to try and replicate the fluorescent emission from plant canopies. A model was located that properly incorporated issues in fluorescence. This model was utilized to determine the canopy level fluorescence of all six soybean conditions. In order to reduce the canopy effects from lower leaf sides a nadir point of view was utilized.
At this point the expected canopy fluorescent signal of a healthy and stressed plant in the field was determined. Calculations were then made in order to determine how much of this signal was lost at a remote detection sight. This was done through the use of radiometric calculations.

The radiometric calculations necessitated the need for information on the atmosphere and plant reflectivity. This information was needed to model the amount of fluorescent signal transmitted through the atmosphere. It was also needed to determine how much background signal was present due to reflected solar and sky light. These values were calculated utilizing the Lowtran (atmospheric model) and Prospect (plant reflectivity model) routines.

The characteristics of each sensor system were included in the radiometric calculations. The determined fluorescent signal at each sensor height incorporated each system’s specific response. The resulting fluorescent signals were then compared to determine if changes in plant condition could be distinguished with such a system.

This was done by taking the ratio of fluorescence at bands 690 nm and 730 nm. The resulting index values, from these ratios, were compared to determine if the difference in plant condition was evident with the remote collection system.

The preceding steps show if it is possible to detect stress at a remote platform. It pursued the hypothesis that stimulated fluorescent emission, from a plant canopy, can be remotely measured in the form of band ratios to indicate plant condition.
2. Background

Prior to determining a specific collection scenario several different aspects of plant fluorescence had to be reviewed. This involved an extensive look into prior research in the field. What follows is a synopsis of that review. It includes different collection methods, factors affecting the fluorescent signal, and fluorescent changes due to different stress conditions.

2.1 PLANT FLUORESCENCE

Fluorescence occurs when light energy is absorbed, by an object, at one wavelength and re-emitted at a longer one. Plants are one type of object that exhibit the phenomenon of fluorescence. In Figure 1 [Lichtenthaler et al. 1988] a typical plant absorption curve is shown.

**Figure 1: Absorption and Chlorophyll Fluorescence Spectra**

![Absorption and Chlorophyll Fluorescence Spectra](image)

Absorption (-) and chlorophyll spectra (—) of a green *Ulmus* leaf. Excitation (470±30 nm) and sensing of the fluorescence were performed from the upper leaf side at steady-state conditions.

[Lichtenthaler et al. 1988]
Also present is the emitted fluorescence curve (excitation at 470 nm ± 30nm) from the chlorophyll portion of the plant. This illustrates that a plant will emit energy at a wavelength longer than the originally absorbed energy.

Plant fluorescence comes from absorbed light energy that is not used by a plant. When vegetation absorbs light it is used in the photosynthesis process or dissipated as heat and fluorescent light. The amount of energy used in photosynthesis depends on the condition of the mechanism in the plant. In a healthy plant 2 to 5% of the absorbed energy is converted to fluorescent light [Lichtenthaler et al., 1988]. If stresses are present, inhibiting the photosynthetic reaction, heat and fluorescent emission rise. In a completely blocked photosynthetic system about 12% of the absorbed light is emitted as fluorescence [Rosema et al., 1991]. By monitoring the amount of fluorescent emission one can detect the presence of plant stress [Lichtenthaler et al., 1988].

This fluorescent emission can be collected either passively or actively. In a passive system solar flux is relied on to excite the plant to fluoresce. The resulting emitted energy is then collected in the Fraunhofer lines. The Fraunhofer lines are used due to the atmosphere removing the solar flux at these discrete wavelengths. This way the noise introduced by solar flux is reduced in the measurement.

The other method is to actively induce fluorescence through use of an external light source. The light source can emit a wide band of wavelengths or just a narrow few. Typically a laser is used due to its selective excitation and power capabilities. The resulting fluorescence can then be measured at any wavelength of interest. Due to the added benefits of an active system, that is the kind being studied in this research.

2.2 KAUTSKY EFFECT (INDUCTION KINETICS)

One of the first methods used to measure plant characteristics was Kautsky induction kinetics. Typically the plant being observed is allowed to undergo dark
adaptation for a period of time. Then the plant is illuminated with light and the resulting fluorescence measured. The fluorescence is not constant over time. An illustration of this is shown in Figure 2 [Lichtenthaler et al., 1988].

**Figure 2: Laser-Induced Chlorophyll-Fluorescence Induction Kinetics**

![Graph of Laser-Induced Chlorophyll-Fluorescence Induction Kinetics]

Laser-induced chlorophyll-fluorescence induction kinetics (Kautsky effect: fast rise and slow decline) upon illumination of a dark-adapted green leaf. The fluorescence rise from the level 0 (constant fluorescence) via I and D to P and the decline via S and M to the steady-state fluorescence T is only found in photosynthetically active plant tissue. [Lichtenthaler et al., 1988]

The curve, referred to as the OIDP curve, starts with an initial fluorescence (Fo) when the light is first turned on. It then begins to rise to a maximum fluorescence value (Fmax) and then subsequently decreases to a steady fluorescent state (Fs). It has been found that this induction curve illustrates much about the ongoing photosynthetic process within a plant [Renger et al., 1986]. This is often done by using various components of the OIDP curve for analysis. Two such examples are the variable fluorescence (Fv) and the fluorescence decrease ratio (Rfd). The variable fluorescence measures the rise from the Fo (initial fluorescence) to Fmax (maximum fluorescence) and indicates the condition of the photosynthetic mechanism. The Rfd is a ratio of the decrease from Fmax (maximum fluorescence) to Fs (steady state fluorescence) and the actual value of
Fs. The Rfd value approximately gauges a plant's possible photosynthetic activity [Lichtenthaler et al., 1988]. Using these and other indicators the induction curve can indicate the physiological state of the plant. Such environmental factors as temperature, water, salinity, and air pollution can cause stress in a plant that will affect it's fluorescent kinetics in different ways [Renger et al., 1986].

There has been a significant amount of research done on Kautsky induction kinetics. The work has centered on showing the effects of different types of stress on the kinetics. However, there are two main problems for use in a remote system. The first being the requirement of predarkening the vegetation prior to measurement. This necessitates using a night time collection. The other requirement is time. In order to collect the entire induction curve it takes on the order of 2 to 5 minutes. This time requirement makes a quick remote collection for wide areas impossible. The first part of the curve may be of use as it reaches it's fluorescent maximum within 100 to 500 ms [Lichtenthaler et al., 1988]. This would allow the initial (Fo) and maximum fluorescent (Fmax) values to be collected from the induction curve when using a remote system. These values of the curve have shown indications of certain plant conditions. While the values Fo and Fmax allow quick collection of data in the field the fluorescent rise is fast and difficult to analyze [Methy et al., 1994]. For this reason, this information does not provide the best insight into plant condition when viewed at a remote level.

2.3 SPECTRAL FLUORESCENCE

In the past most of the research has used some form of the Kautsky induction curve. This information is usually only collected at one wavelength. More recent work has expanded into studying the spectral fluorescent intensity of a plant. The studies have involved trying to assess what spectral parameters are a function of physiological plant condition.
In this case the fluorescence of the plant is measured over a wide range of wavelengths. The spectral fluorescent intensity is typically measured when the plant is in the steady fluorescent state (Fs) of the induction kinetic curve.

2.3.1 Excitation and Collection Wavelengths

The research has been conducted using a variety of excitation wavelengths to create a fluorescent signal that is collected over a broad range of wavelengths. The excitation wavelengths have ranged from 280nm to 632.8nm. The collection band tends to be between 600 and 800 nm, although, more recent work has measured to 400 nm and below by exciting a plant with a UV laser. The results from viewing these returned spectra have indicated several peaks in the output. In an attempt to extract information that relates directly to plant condition different band ratios have been used to try and find a link to plant physiological parameters. The main ratio researched in this effort is the ratio of the fluorescent bands at 690 nm and 730 nm. These wavelengths are used due to the fluorescent emission peaking at these points (see emission curve in Figure 1).

It has been determined that the fluorescent spectra in this area along with the maxima (690 nm and 730 nm) are related to a plant’s chlorophyll content. There are two types of chlorophyll (a and b) that create the two main peaks in this part of the spectrum [Lichtenthaler et al., 1988]. The condition a plant is in affects the relationship between the two chlorophyll levels and thus affects the output fluorescent maximum. The factors influencing the 690/730 nm ratio range from the plant species itself to the many stresses it may be experiencing. What follows is a discussion about what effect various parameters have on the overall fluorescent output and fluorescent ratios such as 690/730 nm.

It should be noted that the two maxima in the fluorescent spectrum are not always located at 690 nm and 730 nm. Depending on the situation the peaks will be located within five or so nanometers. This is due to different plants and conditions. Therefore
researchers tend to follow and report the true maximum from the output. However, the maximum values will be reported at the central peak wavelength and reasonable shifts ignored (ex. 682 reported as 685 nm). For this reason many researchers report slightly different ratios (ex. 685 nm/730 nm vs. 690 nm/730 nm). These ratios typically provide the same information from a plant. This is due to the fact these values are usually collected with bandpass filters that have a bandwidth of 10 nm [Lang, et al., 1994]. Therefore, for our purposes, these ratios will be considered as the same.

2.3.2 Effects of Chlorophyll Concentration

The returned fluorescent signal has been found to be affected by many parameters. The influences of excitation strength and wavelength, ambient light, plant species, age of leaf, time of year, sun or shade leaf, etc. all affect the returned fluorescent signal. Through all these factors the ability to determine a plant's condition is desired. One important parameter in this measurement is the amount of chlorophyll present in a plant.

The fluorescent intensity is dependent on the chlorophyll content of a plant. The higher the content the lower the fluorescence. This is illustrated in Figure 3 [Lichtenthaler et al., 1988].

The figure shows that as a plant matures, and develops more chlorophyll, its fluorescence decreases. This occurs because with increased chlorophyll comes an increase in the plants ability to use the absorbed light in photosynthesis. This also causes the emitted spectra to be altered as a plant ages. The higher levels of chlorophyll cause the peak at 690 nm to decrease more than the peak at 730 nm. This is due to reabsorption of the fluorescent energy at 690 nm by the chlorophyll. This is shown in Figure 1 [Lichtenthaler et al., 1988]. The figure shows that a plant’s absorption peaks in the red portion of the spectrum. Also the fluorescent spectra of chlorophyll is shown to overlap it’s absorption spectra around 690 nm. This allows a plant to reabsorb some of its fluorescent energy. Therefore the ratio of 690/730 nm is also affected by a plants age.

This ratio will go from a value of 1.5, for a young leaf, down to a value of 0.9 to 1.2, for an older leaf. Typically a fully developed leaf with a fully functioning photosynthesis apparatus has values of 0.8 to 1.1 for the adaxial (upper) leaf side.
These values will increase again with the coming of autumn and plant senescence. Senescence is when the functional life of a plant is ended due to deterioration. In perennial plants the leaves die off (undergo senescence) each fall while the roots and stems remain alive [Gausman et al., 1990]. Part of this deterioration process is the breakdown of chlorophyll. As the chlorophyll breaks down the ratio rises from 0.9 - 1.1 to values of 1.5 - 2.0. At this point the leaves can no longer photosynthesize. Further breakdown and color change in the leaf brings the ratio to values of 3 and 4. The fluorescent intensity increases in this process until only 10 μg/cm² of chlorophyll a + b are present. Then the intensity decreases rapidly [Lichtenthaler et al., 1988].

2.3.3 Leaf Side (Upper versus Lower)

Another issue concerning fluorescent measurements is whether the upper (adaxial) or lower (abaxial) side of a leaf is being measured. The lower side of leaves possess less chlorophyll. Therefore the lower side will provide a stronger fluorescent signal with a more prevalent 690 nm peak. This can be seen in Figure 4 [Lichtenthaler et al., 1988].
This figure illustrates the fluorescent spectra from the upper and lower sides of the same type of leaf (sun or shade). In both cases the intensity overall is higher for the lower leaf side. The lower leaf side also has a higher ratio (690 nm/730 nm) value. This affect is present in many different species of plants as can be seen in Table 1 [Lichtenthaler et al., 1988]. The table shows a leaf grown with less light (ex. shade or lower side) has a higher ratio value.
## Table 1: Differences in the Fluorescence Ratio F690/F730 Between the Upper and Lower Leaf Sides of Different Plants

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>F690/F735 upper side</th>
<th>F690/F735 lower side</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Angiosperms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zea mays (C₄ plant)</td>
<td>1.38 (+- 0.08)</td>
<td>1.75 (+- 0.11)</td>
</tr>
<tr>
<td>Avena sativa (equifacial leaf)</td>
<td>1.20 (+- 0.12)</td>
<td>1.32 (+- 0.10)</td>
</tr>
<tr>
<td>Raphanus sativus</td>
<td>0.98 (+- 0.06)</td>
<td>1.59 (+- 0.11)</td>
</tr>
<tr>
<td>Phaseolus vulgaris</td>
<td>0.92 (+- 0.07)</td>
<td>1.70 (+- 0.04)</td>
</tr>
<tr>
<td>Nicotiana tobacum</td>
<td>0.96 (+- 0.11)</td>
<td>1.71 (+- 0.08)</td>
</tr>
<tr>
<td>Fagus sylvatica</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sun Leaf</td>
<td>0.92 (+- 0.11)</td>
<td>1.39 (+- 0.11)</td>
</tr>
<tr>
<td>Shade Leaf</td>
<td>1.06 (+- 0.07)</td>
<td>1.48 (+- 0.06)</td>
</tr>
<tr>
<td>Quercus robur</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sun Leaf</td>
<td>0.90 (+- 0.06)</td>
<td>1.24 (+- 0.08)</td>
</tr>
<tr>
<td>Shade Leaf</td>
<td>0.87 (+- 0.05)</td>
<td>1.41 (+- 0.05)</td>
</tr>
<tr>
<td>Carpinus betulus</td>
<td>0.87 (+- 0.04)</td>
<td>1.30 (+- 0.09)</td>
</tr>
<tr>
<td><strong>Gymnosperms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ginkgo biloba</td>
<td>1.00 (+- 0.06)</td>
<td>1.48 (+- 0.15)</td>
</tr>
<tr>
<td>Taxus baccata</td>
<td>0.69 (+- 0.03)</td>
<td>1.26 (+- 0.09)</td>
</tr>
<tr>
<td><strong>Ferns</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nephrolepis exaltata</td>
<td>1.05 (+- 0.08)</td>
<td>1.64 (+- 0.09)</td>
</tr>
<tr>
<td>Asplenium serra</td>
<td>1.07 (+- 0.07)</td>
<td>1.53 (+- 0.11)</td>
</tr>
<tr>
<td><strong>Livermoss</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marchantia polymorpha</td>
<td>1.32 (+- 0.14)</td>
<td>2.03 (+- 0.16)</td>
</tr>
</tbody>
</table>

*a* Mean values of 12 leaves in each case (with standard deviation).

The differences between the upper and lower leaf sides are highly significant in all plants (P<0.001) with the exception of the equifacial Avena leaf (P = 0.02). [Lichtenthaler et al., 1988]

It has been found that the difference in the 690 nm signal from the abaxial and adaxial surface of the leaf varies greatly from species to species. However, at 440 nm the ratio of the fluorescent signal from opposite leaf sides remains more stable between species. This is possibly due to a property of the stomatal epidermis [Bongi et al., 1994]. The epidermis are the second layers on the top and bottom of a leaf. They are usually
only one cell layer thick and are covered by the waxy cuticle layer. These layers act to help protect the plant and contain the stomata that control water transpiration from the plant [Moon et al., 1960].

2.3.4 Sun/Shade Leaves

Another issue is whether a leaf grew in the sun or shade. This adds to the variability in the fluorescent signature for a certain plant species. The difference in development light causes physiological differences in the leaves. Leaves grown in the sun typically have more chlorophyll content than shade grown leaves. These differences show themselves by the shade leaves having higher fluorescent intensities. Figure 4 [Lichtenthaler et al., 1988] shows this exact variation in a beech leaf. The differences in the ratio 690/730 nm are not large but are certainly different for the upper side of the leaf, see Table 1 [Lichtenthaler et al., 1988].

2.3.5 Species Variation (Monocots/Dicots)

The species to species variability also affects the fluorescent signal. The two major plant groups of monocots and dicots both act differently. These are two major subclasses of flowering plants (angiosperms). In angiosperms a unique leaf develops in the seed as the plant begins the growth process. This initial leaf provides needed nutrients to a plant in it’s developing stages. In monocots only one of these original leaves is formed. In dicots two leaves are developed from the seed’s sprout [Moon et al., 1960]. When exposed to 340 nm light, monocots emit strongly at 440 nm with a small shoulder at 530 nm. Dicots on the other hand have a less intense value at 440 nm and a more prominent 530 nm intensity. Therefore the ratio 440/530 nm shows the difference between the two groups. Also when excited at 280 nm both dicots and monocots exhibited high 340 nm fluorescence but only monocots showed a maximum at 440 nm
[Corp et al., 1994]. This difference in species fluorescence can be seen in the ratio 340/440 nm. This illustrates the variability in fluorescence for different species.

2.4 PLANT STRESS

These are just some of the parameters affecting plant fluorescence. There are many stresses that a plant can undergo that will affect its fluorescent emission. While fluorescent ratio 690/730 nm will increase at lower chlorophyll levels, changes in the value also show other factors. The ratio 690/730 nm also increases when a plant's quantum conversion processes in photosynthesis is inhibited. Therefore the ratio 690/730 nm will indicate both lower chlorophyll content and a weakening photosynthesis process. Both these issues can show stress in vegetation. The actual effects of specific stresses will be discussed in the following paragraphs. In general, it has been noted that a value of 1.1 for the ratio 690/735 nm shows beginning stress while values above 1.2 show definite stress [Lichtenthaler et al., 1988]. Generally fluorescence increases with stress level. Figure 5 [Lichtenthaler et al., 1988] illustrates that by adding stress (Herbicide DCMU) the fluorescent intensity increases.
The following discussion contains information on different stress conditions (including herbicide stress) and their various effects on plant fluorescence.

### 2.4.1 Herbicide Stress

In agricultural farming herbicides are often used for pest control. The problem is that herbicides can inhibit the photosynthetic reaction once they are absorbed by the plant. DCMU is one type of herbicide that inhibits the photosynthetic process. It does this by blocking the reaction that produces oxygen from the splitting of water molecules [Chappelle et al., 1984a]. The more they block the photosynthetic process the more fluorescent emission is present. This increase is accompanied by a change in the 690/735 nm ratio. The 690 nm band will increase up to 2.9 times it's original value. The 730 nm band only rises 2.3 times (excitation 470 +/- 30 nm) [Lichtenthaler et al., 1988]. This
change in the ratio is higher for the upper leaf side. This is due to the fact that the lower side already has a higher peak at the 690 nm band. The resulting spectra from herbicide contamination is similar to that of young leaves still greening with age. An example of radish seedlings undergoing herbicide stress is shown in Figure 5 [Lichtenthaler et al., 1988]. It illustrates the above mentioned results of herbicide stress on the fluorescent spectra. Changes in the 690/735 nm ratio for different species undergoing DCMU stress can be seen in Table 2 [Lichtenthaler et al., 1988].
### Table 2: Differences in the Chlorophyll-Fluorescence Ratio F690/F735 in Leaves of Different Plants After DCMU Treatment and in Needles of Healthy (Damage Class 0) and Damaged Conifer Trees (Damage Class 2 or 3)

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Ratio F690/F735 of upper leaf side</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Nicotiana tabacum</em></td>
<td>Control: 0.97 (+- 0.070), +DCMU: 1.18 (+- 0.05)</td>
</tr>
<tr>
<td></td>
<td><em>Raphanus sativus</em></td>
</tr>
<tr>
<td></td>
<td>Control: 0.98 (+- 0.09), +DCMU: 1.33 (+- 0.13)</td>
</tr>
<tr>
<td><em>Quercus robur</em></td>
<td>Control: 1.31 (+- 0.12), +DCMU: 1.83 (+- 0.15)</td>
</tr>
<tr>
<td><em>Albies alba</em></td>
<td>Healthy (class 0): 0.95, Healthy (class 3): 1.45</td>
</tr>
<tr>
<td></td>
<td>Damaged (class 3)</td>
</tr>
<tr>
<td><em>Picea abies</em></td>
<td>Healthy (class 0): 0.97, Healthy (class 2): 2.16</td>
</tr>
<tr>
<td></td>
<td>Damaged (class 3)</td>
</tr>
<tr>
<td><em>Pseudotsuga menziesii</em></td>
<td>Healthy (class 0): 0.97, Healthy (class 0): 0.97</td>
</tr>
</tbody>
</table>

---

**Note:**

- Mean values of 10 leaves with standard deviation
- Mean values of 5 leaves; maximum deviation 12% or less. The differences between control and DCMU-treated leaves as well as between healthy and damaged needles are significant (P = 0.001). [Lichtenthaler et al., 1988]

In the case of pine needles the addition of the herbicide DCMU causes the appearance of a 685 nm band and an increase in the 730 nm and blue fluorescence (with excitation 337nm) [Chappelle et al., 1987]. Typically coniferous trees are very efficient in their photosynthetic process. Therefore in healthy conditions the 685 nm peak is not
very prominent in the fluorescent spectrum. Only when undergoing stress does a coniferous plant begin to exhibit a peak in fluorescence at 685 nm.

2.4.2 Daylight Cycle (Short Term Days)

Plants are accustomed to the natural day(light) night(dark) rhythm. After prolonged exposure to a dark environment the chlorophyll begins to break down. This is one of the reasons conifers have less chlorophyll in the spring following the darker periods of winter. The effect this has on fluorescence is a rise in the intensity when subjected to prolonged darkness. The 690 band increases more relative to the 730 band. Therefore ratio values increase to 1.5 (from 0.9 to 1.1) and even past the value 2 [Lichtenthaler et al., 1988].

2.4.3 Water Stress

One of the most commonly researched factors affecting plant condition is water availability. Water is very important for plant growth as it is involved in several different reactions. One of water's main uses is being the primary electron donor in the photosynthetic reaction [Chappelle et al., 1987]. A lack of moisture therefore hampers the photosynthetic process. Plants that are prone or tolerant to drought provide a similar fluorescent pattern to water stress when measuring the Kautsky induction kinetics [Karukstis et al., 1991, Renger et al., 1986].

One may hypothesize this similar response in Kautsky induction kinetics indicates the following statement: some plants with different tolerances may have a common steady state fluorescent spectrum signature for water stress. This will depend on the situation. It must be kept in mind that plants adapted to different conditions respond differently to water stress. The main difference being tolerant plants have the ability to recover from water loss [Renger et al., 1986]. The different adaptations may cause
variances in the returned fluorescent spectrum. It has been shown that varying species do have different fluorescent spectrums in the face of drought.

Plants undergoing long term drought, that contain broad leaves or are of the annual variety, tend to have a decreasing intensity in the fluorescent signal. The intensity of the signal varies when the water stress is less than 24 hours. This was determined in a study that detached plant leaves and measured their fluorescent output until they were thoroughly dried. It illustrated that depending on the water content of the plant the fluorescent intensity will sometimes rise. The 690/730 nm ratio will however consistently increase from the beginning of water deprivation. A tobacco leaf (90% water) when illuminated with 470+30 nm will go from a control of 0.93 to a value of 1.25 after 24 hours and then up to 1.86 with more drying. Beech leaves (72% water content) go from a value of 0.9 to 1.1 after 24 hours of drought [Lichtenthaler et al., 1988]. Conifers, while having the same trend in the 690/730 nm ratio, present a different trend in intensity values. The effect of water stress is much slower on conifers. As a result the fluorescent intensity decreases for the first 30 hours followed by an increasing fluorescent signal with continued drought [Lichtenthaler et al., 1988]. Healthy conifers do not have a 685 nm band when excited with 337 nm. However, this band does appear once the plant is water stressed [Chappelle et al., 1987].

The excitation wavelength used in part dictates what information is received from a water stressed plant. While the overall fluorescent intensity (of Quercus pubescens, oak) decreases with drought when using wavelengths 308, 355, 480 nm for excitation, each spectrum is different. In the case of 308 nm and 480 nm a peak at 710 nm and 713 nm respectively appears for each of the two excitation wavelengths [Cecchi et al., 1994a, Cecchi et al., 1994b, Valentini et al., 1994]. However, when using a 355 nm excitation wavelength the ratio of 685/730 nm is comparable for the healthy and stressed plant (0.35:healthy vs. 0.3:stress) [Gunther et al., 1994].

36
Another issue with water stress appears to be the time of day of the collection. Increasing sunlight further hampers an already water deficient plant. Therefore the differences are more pronounced during high daylight hours (afternoon). This was seen for both the populus alba L. and the Quervus pubescens (oak) [Valentini et al., 1994]. This causes the normal relationship between ambient light and fluorescent intensity to change for a water stressed oak (excitation 355 nm). The change is, the intensity of fluorescence decreases with decreasing ambient light [Gunther et al., 1994]. The ratio 690 nm/730 nm, in this case, tends to increase with lower light levels [Valentini et al., 1994].

Another condition to keep in mind is the adaptation of a plant to its surroundings. An olive leaf (o.europa) when grown under high light drought conditions will have 5 times more epicuticular waxes than a normal leaf. This is a wax that forms on the outer surface of a plant leaf and protects the plant from water loss. The increase in wax shows itself with a fluorescent signature at 440nm that is 3 times the normal value when excited with 337 nm. When grown in low light drought conditions the fluorescent increase is less noticeable [Bongi et al., 1994]. It appears, therefore, when looking at the fluorescent signature for drought stress one has to consider the ambient light condition and the excitation wavelength used.

2.4.4 Temperature

The temperature of a plant can provide unwanted stress on the photosynthetic process. When taking a plant at room temperature (20 to 25° C) and then measuring fluorescence at low (5 to 10° C) or high (30 to 40° C) temperatures there is no change in the spectrum or intensity. However, exposure to these temperatures for longer periods of time does affect a change. The frozen leaf (-17° C) of a Prunus laurocerasus showed a decrease in the overall fluorescence when excited at 470+-30nm (18° C). In this case, the ratio of 690/735 nm did not significantly change. This response was similar to that of
a partially hydrated leaf [Lichtenthaler et al., 1988]. In fact fluorescence in the 690 nm band only rises for an extreme rise in temperature at which point the plant is damaged. The 440 nm band shows more of a trend with temperature. It has been shown that a Lolium perenne in darkness has an inverse relation between the fluorescence at 440 nm and the temperature between 6 and 28°C. This is with an excitation of 337 nm. It was also shown that the sensitivity of fluorescence at 440 nm to temperature depended on the species [Bongi et al., 1994].

The best way to measure temperature stress, besides the use of the 440 nm band, is to measure the induction kinetics of fluorescence. The effects of high and low temperature stress affect the kinetics differently and therefore allow better resolution of change in plant condition. Typically high temperature causes the initial fluorescence (Fo) and variable fluorescence (Fv) to rise [Karukstis et al., 1991, Renger et al., 1986]. Chill stress on the other hand does not affect "Fo" but causes "Fv" to decrease. It also changes the rise of the OIDP induction curve [Karukstis et al., 1991, Renger et al., 1986]. The effects of temperature stress can be altered depending on the presence or absence of light stress [Karukstis et al., 1991]. So far the analysis of temperature stress appears best done with Kautsky induction kinetics.

2.4.5 Mechanical Injury

Injury to the plant through mechanical stress alters the fluorescent signal. The mechanical pressure causes the intercellular spaces in the plant to become reduced. This causes the items such as chloroplasts to become more densely spaced. Therefore more of the fluorescent light is reabsorbed and less intensity emitted. This affects the 730 nm band more than the 690 nm band. Therefore the ratio of the bands increases, as seen in maize (zea mays 1.13 to 1.74) and radish (raphanus 0.96 to 1.76) plants illuminated with 470+-30 nm [Lichtenthaler et al., 1988]. Of course the amount of pressure dictates the amount of injury and change in fluorescent signal.
2.4.6 Nitrogen

The amount of nitrogen a plant receives influences its ability to function. Plants deficient in nitrogen have a lower chlorophyll content. This results in an increase in the fluorescent intensity and 690/735 nm ratio due to lower reabsorption. Eventually a severe lack of nitrogen will cause chlorophyll breakdown and the signal will resemble that of a plant undergoing senescence. If plant condition is in question multiple measurements may be needed. Plants typically adapt to lower nitrogen levels by developing sun type chloroplasts which are more efficient. This is seen by measuring the Rfd value from Kautsky induction kinetics [Lichtenthaler et al., 1988]. Therefore if nitrogen levels are only slightly lower than normal the adaptability of the plant will allow it to still function well.

Nitrogen levels have an effect on the plant as a whole, not just the chlorophyll. As a result much of the fluorescent spectrum is influenced. When soybean plants are excited with 280 nm the output at 340 nm decreases with lower levels of nitrogen [Corp et al., 1994]. Field corn (Zea mays L.) is a plant that has undergone much research with varying fertilization. In looking at corn with over and under fertilization, fluorescence can discern high from low levels of nitrogen. Typically the overall intensities of fluorescence at 440, 525, 685, and 740 nm (using excitation at 337 nm unless otherwise noted) only discern the highest from the lowest nitrogen levels. By creating a ratio from these bands more information was found. Using the ratio 440/685 nm the 100% and 150% levels of optimal nitrogen could be separated from the 50% and lower levels [25.22 (100%) to 8.71 (0%)] [McMurtrey III et al., 1994]. The ratios 525/685 nm [11.14 (100%) to 4.44 (0%)] and 740/685 nm [1.64 (100%) to 0.79 (0%)] were able to discern the top two levels from the 75% and below levels [McMurtrey III et al., 1994]. In fact the 740 nm value was the lowest at 100% optimal fertilization. This gives promise as to the ability to differentiate improper levels of fertilization, both high and low. These
results appear to be in question with some earlier work done by the same author but the trend in the 740/685 nm ratio remains and differentiating different levels of nitrogen appears feasible [McMurtrey III et al., 1990].

2.4.7 More Nutrients

Improper levels of other plant nutrients may also cause changes in a plant's fluorescent signature. Excess or deficient levels of important plant nutrients can affect the development of chloroplasts and/or block it's photosynthetic function [Karukstis et al., 1991]. Which is affected, photosynthesis or chlorophyll development, depends on the nutrient. Which nutrients are required depends on the plant type. This can be seen in two separate studies; one on corn and one on rice plants. In the corn study seven nutrients were used at different levels. Using an excitation wavelength of 337 nm it was found that fluorescence at 440 nm decreases from normal conditions when plants are deficient in potassium, phosphorous, nitrogen, or iron. The better indicator was the 685 nm band, as potassium deficiency raised fluorescence to three times the control level. Also nitrogen and iron low soils caused the intensity to decrease to half the control value [Chappelle et al., 1987]. In contrast, the study on rice did not see a three fold increase in potassium low stress at 690 nm. What was seen were peaks at 690, 705, 725, 745, 750 nm. All deficiencies in this case caused increased fluorescence at 690 nm which was not the case for corn. The highest increase was for nitrogen and magnesium. However, a reason the two studies may not correlate is that in the rice study an excitation of 632.8 nm was used to illuminate the lower side of the plants leaves. The high resolution of the recording spectra also identified the extra fluorescent peaks [Subhash et al., 1994].

These peaks may allow differentiation between nutrient stresses. The 705 nm band was high for plants lacking in silicon, magnesium and iron. The 725 nm band was high for silicon and magnesium while being low for nitrogen deficiency. The 745 and 750 nm bands were highest for silicon and lowest for nitrogen deficiency. The trend for
the bands 725, 745, and 750 nm were similar. The 690 and 705 nm bands were also similar except for silicon deficiency. This indicates creating a ratio from various spectral peaks may allow differentiation of nutrient deficiencies. Using the Fmax values from the induction curve, the ratios 690/705 nm and 690/725 nm were looked at. The maximum ratio for 690/725 nm was for phosphorous (1.846 compared to control of 1.121). The band 690/705 nm had a high for nitrogen (1.655 compared to a control of 1.094) [Subhash et al., 1994]. Comparing these results to ratios taken at steady state fluorescence may not be possible. The assumption that the ratio of bands taken at fmax and steady state fluorescence values are similar is not necessarily true. As a plant’s fluorescence drops from Fmax to the steady state it’s spectral output may change. For this reason care has to be taken as to the plant’s fluorescent state when ratios are compared. While these parameters, such as excitation wavelength and plant species, need to be taken into account identifying the presence of nutrient stress appears possible. Taking the ratio of different bands may even allow separation of different nutrient stresses.

2.4.8 Senescence

The coming of autumn also places the plant under stress. The chlorophyll begins to breakdown and the photosynthesis process declines. As mentioned earlier this causes healthy plants with a 690/735 nm band ratio of 0.9 - 1.1 to increase to 1.5 2.0, for yellowish green leaves. Further yellowing and chlorophyll breakdown brings the values up to 3.5 - 4.0. They can even reach a value of 8.5, such is the case for the yellowish parthenocissus leaves (containing 0.5 to 1.0 μg/cm$^2$ of chlorophyll). These values are similar for any stress that causes the plant's chlorophyll to breakdown (not just senescence). The start of this process can be seen in the plant's fluorescent signature in August prior to chlorophyll breakdown. This is especially evident when looking at the kinetic induction curve values [Lichtenthaler et al., 1988].
2.4.9 Soil Salinity

Salinity in the soil is a problem for plants. This becomes an issue in areas using a lot of irrigation or near high evaporation areas by salt water bodies. Levels of salt can become toxic for a plant when they are too high. The level of salt depends on the particular plant's tolerance. Eventually the limit a plant can handle is reached and it's fluorescent kinetics are affected [Lichtenthaler et al., 1988]. This is due to the photosynthetic process being limited. It has been found that the OIDP rise of induction kinetics can be used to find plant tolerance to salt stress [Karukstis et al., 1991]. Eventually long periods of salt stress will cause a plant's chlorophyll to breakdown. The fluorescent signature is then similar to a plant undergoing autumnal senescence [Lichtenthaler et al., 1988].

2.4.10 High Ambient Light Levels

Light intensities that are much higher than normal for a plant will cause photoinhibition [Renger et al., 1986]. Photoinhibition is caused by high light levels depleting some of the compounds needed in the photosynthetic process. As a protective device more of the excitation energy is dissipated as heat [Renger et al., 1986]. Therefore the variable part of fluorescence kinetics is removed. The induction curve no longer reaches Fmax. Instead the initial fluorescence, Fo, is reached and levels off to a constant fluorescence. The intensity of this constant fluorescence is similar to the steady state fluorescence of a fully intact leaf. This basically produces a flattened induction curve. Photoinhibition mainly occurs in mature leaves and is enhanced by high heat and water stress conditions. Being exposed to infrared light makes a plant further susceptible to this stress. Once the plant is removed from the intense illumination it will eventually recover to normal fluorescence levels [Lichtenthaler et al., 1988].

42
2.4.11 UV-B Radiation

A form of light stress that is of growing concern is the increase of UV-B radiation due to the diminishing ozone layer [Renger et al., 1986]. It has been found that long term exposure to UV radiation causes a decrease in blue fluorescence (450 nm) and an increase in chlorophyll fluorescence (685 and 730 nm). This was found for a Salvia spenden leaf excited at 337 nm. The green band at 536 nm remained the same intensity when exposed but the peak shifted 10 nm towards shorter wavelengths. It is thought that the UV radiation alters the flavonoids (pigments that are UV protecting) in the leaf's epidermis. This transition tends to lower the absorbing ability (in the UV band, 450 nm) of pigments in the epidermis. This allows deeper penetration of the excitation light into the leaf thus exciting more chlorophyll. This leads to higher chlorophyll fluorescence [Mazzinghi et al., 1994].

2.4.12 Air Pollution

The quality of air surrounding a plant can affect its condition. The elements of CO₂, NH₃, and ozone have all been shown to influence a plant's condition. The effect air pollution has on a plant depends on the pollutant but it will in some way influence the photosynthetic capacity. Ozone, SO₂, and high levels of CO₂ have all been found to decrease the variable fluorescence (from Kautsky induction kinetics) [Renger et al., 1986, Karukstis et al., 1991]. On the other hand, low levels of CO₂ only cause an increase in Fo and combined contaminants of SO₂ and NH₃ appear to counteract each others effects [Karukstis et al., 1991]. This supports the notion that the influence air pollution has on a plant, and its fluorescence, is complex in nature. It is known, however, that long term exposure to ozone will lower a plant's chlorophyll and cause higher 690/730 nm ratio values [Snel et al., 1994].
2.4.13 Acid Rain

Acid rain damage also shows up in the fluorescent signature. Damaged red spruce (*Picea rubens* Sarg.) and Norway spruce when excited with 337 nm show a peak at 685 nm [Chappelle et al., 1987, Banninger et al., 1990]. Typically this fluorescent peak (at 685 nm) is not present in healthy conifers. Also evident in the spectrum is a relation between the fluorescent intensity at 440 nm and degrees of tree damage due to acid rain. In fact it was a better indicator than the ratio 440/740 nm [Chappelle et al., 1987]. Both the 440 nm and 525 nm band decrease in intensity with increasing tree damage [Chappelle et al., 1987, Banninger et al., 1990].

2.4.14 Metals

Metals located in the soil produce adverse affects on plants as well. The influence on the fluorescent signal of Norway spruce has been shown but with too much variability. This variability was due to needle age and time of year when the measurement was made [Banninger et al., 1990]. Reducing these factors would be necessary to draw conclusions as to the effect of metals on plant fluorescence.

2.4.15 Summary

The above discussion indicates how different stress factors affect fluorescence. It is evident that some of these factors have undergone more research than others. In general increasing the stress of a plant increases it’s fluorescent intensity. Stress also has profound effects on the fluorescent spectrum. Typically an increase in the ratio 690/730 nm accompanies a stress factor. A value of 1.1 tends to show beginning stress while a ratio of 1.2 indicates definite stress [Lichtenthaler et al., 1988]. In some cases Kautsky induction kinetics appears to be a better indicator of certain types of stress.

The information presented was used to determine what stress type was best to use in this research. Some of the following criterion were used in this decision: amount of
past research, stress indicated by ratio 690/730 nm, and plant type used in past research along with stress. After the stress type was chosen, the collection parameters effects on the fluorescent signal had to be determined.

2.5 PARAMETERS FOR COLLECTING PLANT FLUORESCENCE

In order to analyze the health and stress signals from plants the system specifications used for collection need to be considered. When collecting the fluorescent signals of plants there are several parameters that affect the returned signatures, the first being the characteristics of the excitation light.

2.5.1 Excitation Intensity

It has been shown that fluorescence decreases linearly with lowering excitation intensities. This variance does not affect the overall spectrum of fluorescence. As excitation light levels are altered the ratio 690/730 nm remains the same [Lichtenthaler et al., 1988].

2.5.2 Excitation Wavelength

The wavelength used for excitation also has some significance. While several different wavelengths have been used to try and determine plant condition, not all of them produce the same fluorescent signal. It has been noted that excitation wavelengths in the range from 400 to 500 nm provide similar results while wavelengths between 525 nm and 600 nm change the returned spectrum significantly [Lichtenthaler et al., 1988]. This trend was indicated for the upper side of a sun grown beech (Fagus sylvatica) leaf. The fluorescence of the beech leaf at different excitation wavelengths is shown in Figure 6 [Lichtenthaler et al., 1988].
Chlorophyll-fluorescence emission spectra of a green sun leaf of the beech (*Fagus sylvatica*, upper leaf side) using excitation light of different wavelengths. The measurements were performed at steady-state fluorescence. The wavelength range selected in this case was \( \lambda \) max ±10 nm at 1 = 400 nm, 2 = 425 nm, 3 = 450 nm, 4 = 470 nm, 5 = 500 nm, 6 = 525 nm, 7 = 550 nm, and 8 = 600 nm.

The relative independence of the fluorescent spectrum, when excited in the range from 400 nm to 525 nm, was also illustrated for a soybean plant. It was noted that while the relationship between bands 685 nm and 730 nm remained the same, the overall fluorescent intensity did change with excitation wavelength. In the beech leaf study the change in fluorescent intensity was attributed to changing excitation intensities. In another study on soybean it was found that the fluorescent intensity did change with wavelength. In this case the excitation wavelengths were altered while keeping the same overall excitation intensity. Figure 7 shows the fluorescent intensity changes in soybean with different excitation wavelengths [Chappelle et al., 1987].
This plot illustrates that fluorescent intensity does change with wavelength. It also shows that using excitation wavelengths ranging from 337 nm to 440 nm or 525 nm to 640 nm produce a slight change in the relationship between the 685 nm and 740 nm fluorescent bands. The use of excitation bands above 640 nm causes the 690 nm band to disappear. Also the 440 nm band no longer appears when illuminating with wavelengths above 410 nm [Chappelle et al., 1987]. Excitation wavelengths between 400 nm and 525 nm provide similar trends in soybean fluorescence at 685 nm and 740 nm.

Due to the altering of the signal, special attention needs to be paid to the excitation wavelength used. Both studies (beech and soybean) show that illumination wavelengths between 400 nm and 525 nm appear the best choice for providing consistent 690/730 nm ratios.
2.5.3 Ambient Light

Typically the research conducted has been on single leaves in a controlled lab environment. When trying to make vegetation assessment using the whole plant, in a greenhouse or field, other factors enter the picture. Items such as changing ambient light and effects of canopy structure are influences on the returned fluorescent signal.

It has been shown that fluorescence is greater in the dark versus sunlight conditions (see Figure 8)[Cecchi et al., 1994a].

![Figure 8: Red Fluorescence Spectra in Different Sunlight Conditions](image)

This is due to the link between photosynthetic activity and fluorescence. In the dark a plant is less prepared to utilize incoming light. Therefore if more excitation light impinges on the plant more of it is released as fluorescence. The effect of increasing photosynthetic photon flux density (PPFD) is a decrease in fluorescence. This is due to the photosynthetic process becoming more efficient with increasing light levels. This decrease is especially prevalent in the 685 nm band (using excitation at 480 nm). The effects of ambient light on the ratio of the fluorescent bands 685 nm and 730 nm is not always the same.
In many cases the ratio 685/730 nm (RFR index) decreases with higher ambient light levels (see Figure 9) [Valentini et al., 1994].

Figure 9: Diurnal Behavior of the RFR Index (685/730 nm) and of the PPFD

Diurnal behavior of the RFR index and of the photosynthetic photon flux density (PPFD). The PPFD was measured close to the fluorescence detection point. [Valentini et al., 1994]

In fact the ratio 730/685 nm (from a maize plant with excitation at 480 nm) is said to be linear with PPFD and the net photosynthesis [Cecchi et al., 1994b]. The ratio 730/685 nm is used by some researchers due to it providing a linear relation to physiological parameters instead of the inverse relation the standard ratio of 685/730 nm provides. This linear trend with PPFD and the ratio 730/685 nm was also shown to be correlated with leaf temperature. The standard ratio of 685/730 nm (from a Juglans regia L.) was found to decrease from a value of 0.98 to a value of 0.65 during maximum sunlight (1500 μmol/m²s) [Mazzinghi et al., 1994].

However the change in the ratio does not appear to always be from the same band (690 or 730 nm) changing its fluorescence. In one study Quercus ilex and Populus alba were excited with 633 nm light under different ambient light conditions. The ratio 690/730 nm was found to decrease 25% for Quercus ilex and 18% for Populus alba when light conditions went from 0 to 2000 μmol/s/m². The decrease in the ratio, with
increasing light, was attributed to a larger decrease in the 685 nm fluorescence than the 730 nm fluorescence. This change in each bands contribution to the fluorescent signal is illustrated in Figure 10 [Valentini et al., 1994].

![Figure 10: Changes of the Relative Contribution of the F690 and F730 Bands to Total Fluorescence (Over Varying PPFD)](image)

\[ F_t = F690 + F730 \]  
\( (Quercus ilex) \)  
[Valentini et al., 1994]

It has also been shown, with 530 nm excitation, that the ratio 685/730 nm from a Douglas fir decreases 20-30% at high PPFD. Another instrument, LEAF, (Laser Environmental Active Fluorosensor- one kind of fluorosensor often used in collection of fluorescent data, utilizes 632.8 nm excitation) was used to measure the Douglas fir in the field at a distance of 30 m with varying ambient light. In this case the band 680 nm was observed to stay relatively constant as the 730 nm increased with higher ambient light levels (see Figure 11, FBR = 680 nm/730 nm) [Snel et al., 1994]. The difference in this relationship (between 690 nm and 730 nm), compared to Figure 10, could be due to the information being collected at a remote location.
At this excitation it was stated that the ratio 685/730 nm depended on chlorophyll content and ambient light.

This is in contrast to another finding that the ratio 685/730 nm is nearly independent of ambient light. Using 355 nm excitation light it was found that the bands 685 and 730 nm increase at the same rate as ambient light decreases. It was also found that a peak at 440 nm increased less. The rates of increase versus lowering ambient light are illustrated in Figure 12 [Gunther et al., 1994].
Figure 12: Daily Cycle of the Fluorescence Signals at 440 nm, 685 nm, and 730 nm

Daily cycle of the fluorescence signals at 440 nm, 685 nm, and 730 nm (left y-axis) of *Quercus pubescens*, and global irradiation (right y-axis) measured in 10 October 1992. 1 μeinstein/(m²s) is equivalent to $6022 \times 10^{13}$ photons/(m²s). [Gunther et al., 1994]

The different rates of change cause the ratio 440/685 nm to linearly decreased with lower light levels. The ratio 685 nm/730 nm in this case is relatively independent of the light level (see Figure 13) [Gunther et al., 1994].

It appears that the specific excitation wavelength used has a definite affect on how ambient light level influences the recorded spectrum. Plant species also seems to play a part in the affect ambient light has on the signal.

The ability to see these signatures from a distance has been researched. Some of the work so far has used Lidar systems at a remote distance to look at plants [Cecchi et al., 1994a]. Lately more work has been done on trying to relate the signature seen at the leaf level to that of a canopy [Cecchi et al., 1994c]. The effects of ambient light at the leaf (excitation 633 nm) and canopy level (excitation 480 nm) were collected and compared. It was found that both demonstrated a decrease in the ratio 685/730 nm with increasing light. While reported for different vegetation, it was found that at near field the ratio was 1.1 at night and 0.75 at maximum sunlight (PPFD = 1800 μmol/m²s). Far field produced a value of 0.95 at night down to a value of 0.6 during full sunlight (PPFD = 1200 μmol/m²s) [Valentini et al., 1994].

53
2.5.4 Modeling of Canopy Effects

When looking at the fluorescent signature of a canopy instead of an individual leaf many influencing factors are added. The density of the canopy (number of branches and leaves), background, and plant orientation have to be considered. The effects of canopy features on reflected light have been modeled [Banninger et al., 1990]. However, a plant canopy affects reflected light and emitted fluorescence energy differently. In fluorescence, leaves act as lambertian emitters and are not as influenced by leaf angle [Barnes et al., 1990]. Recent research has begun to try and determine the appearance of canopy fluorescence by modeling the unique attributes of plant fluorescence.

In one case a method was developed to determine leaf area and height by analyzing the backscattered fluorescent signature from a plant canopy [Barnes et al., 1990]. This helps determine leaf area index (LAI) but does not totally illustrate the many parameters affecting the fluorescent signal emitted by a canopy. Leaf area index is a ratio of the leaf area for a certain ground area [Jordan, 1969].

2.5.5 Canopy Model by Rosema, A.

Recently two models have appeared in the literature that describe the fluorescence signal from a plant canopy. In a recent effort by Rosema et al. (1991) the simulation of both leaf and canopy fluorescence was accomplished through a radiative transfer model. The canopy model was derived from the previously developed reflectance SAIL model [Verhoef, 1984]. This method by Rosema first models the fluorescence of the leaf. The leaf model is an extension of the work by Fukshansky, L. et al. (1980). The output of the revised leaf model depends on the fluorescence quantum efficiency, absorption coefficient (proportional to leaf chlorophyll content), background reflectance, and leaf thickness. The canopy fluorescence is then determined with the FSAIL model. The input parameters necessary are the leaf area index and the leaf inclination of the canopy. The output provides a reasonable expectation of canopy fluorescence. It takes into
account the fluorescence in the upward and downward direction along with absorption and scattering by the leaves. The results illustrated the sensitivity of the signal to chlorophyll content of the leaves and background reflection [Rosema et al., 1991].

2.5.6 Canopy Model by Olioso, A.

The second modeling effort that illustrated the effects of the canopy on a fluorescent signal was completed by Olioso et al. (1992). The model took into account the following factors: the attenuation inside the canopy of excitation energy, leaf fluorescence due to excitation, the attenuation of the emitted signal towards the sensor, and the fluorescent signal reaching the sensor due to energy reflecting off the soil. The attenuation of radiation inside the canopy was assumed to follow Beer-Lambert's law. The fluorescent efficiency of the leaves was assumed to be independent of excitation and the leaf fluorescence lambertian in nature. Also the leaf optical properties along with their angular distribution were assumed to be uniform throughout the canopy. The effects of multiple scattering within the canopy from leaf emission were not included in this model.

The changing fluorescent signal from the inner canopy was also taken into account. Leaves within a canopy receive less light and develop less chlorophyll. This causes the fluorescent efficiency to be higher. Therefore, deeper inside the canopy the fluorescence increases in relation to the amount of excitation energy it receives. This is true more for the 690 nm band of fluorescence than the 730 nm band. The increasing rate of leaf fluorescence with canopy depth for each waveband is illustrated in Figure 14 [Olioso et al., 1992]. The figure illustrates how the fluorescence of soybean leaves (upper side) changes with canopy depth when excited with a 632.8 nm (He-Ne) laser.
Leaf fluorescence profiles following Eq. (9): (--) $a = 0.94$, $b = 0.06$, $c = 0$; (---) $a = 0.94$, $b = 0.06$, $c = 0.7$; (-) $a = 1.36$, $b = 0.20$, $c = 0.20$; experimental points from 690 nm (o) and 730 nm (+) profile D in Figure 2. (see figure reference in paper by Olioso) [Olioso et al., 1992]

This information was used in the determination of the amount of canopy fluorescence. Typically it only adds a small amount to canopy fluorescence. When the canopies are less transparent the effects of deeper layers on the fluorescent signal is limited due to more of the radiation being attenuated. However, as the interception and absorption properties of a canopy become less efficient the deeper layers are more influential in the signal.

How efficient a plant canopy is at intercepting light depends on the canopy type. The following canopy types: erectophile, spherical, uniform, and planophile are in order of increasingly efficient interceptors of light [Olioso et al., 1992]. Their classification depends on the average angle of leaf inclination (ex. spherical (57°) and planophile (10°)) [Rosema et al., 1991]. The effect of the four different canopy types on the resulting canopy fluorescence is illustrated in Figure 15.
Figure 15 shows the canopy fluorescence with and without soil reflection ($\rho$). It also illustrates changes due to altering the inner canopy fluorescence (changing the value "c" located in exponential term described in the procedure).

**Figure 15: Simulations of Canopy Fluorescence**

| Simulations of canopy fluorescence $F_c$ and soil term $F_2 + F_3$: (---) 730 nm; (—) 690 nm; 1) planophile canopy; 2) uniform; 3) spherical; 4) erectophile. | [Olioso et al., 1992] |

The results of the model determined that LAI, canopy structure, and background reflection all affect the canopy fluorescence. Canopy fluorescence was also shown to vary with canopy depth (changing fluorescent efficiency) and four canopy types (see Figure 15). These factors appear to make the analysis of canopy fluorescence difficult to
make without additional information from other measurements. However, the effects of the canopy structure are limited by analyzing the band ratio 685/730 nm. When leaf area index of a plant increases its fluorescent intensity dramatically increases. This is accompanied by a preferential increase in the 730 nm fluorescence. Therefore the ratio 685/730 nm does decrease with LAI. The ratio, however, drops by less than 40% of it's original value as a plant's LAI increases. This drop as LAI increases can be seen in Figure 16 [Olioso et al., 1992].

**Figure 16: Simulation of F_{690}/F_{730} for a Spherical Canopy and Different Leaf Chlorophyll Content**

Simulation of F_{690}/F_{730} for a spherical canopy and different leaf chlorophyll content (the four simulated evolutions for each chlorophyll content value correspond to the four parameter sets in Fig. 6 (see figure reference in article by Olioso). [Olioso et al., 1992]

This figures illustrates four curves for four different levels of chlorophyll concentration (10, 20, 40, 60 μg/cm²). Each set of curves represents different levels of background reflectance and amount of influence from inner canopy leaves. This plot
illustrates that varying chlorophyll amounts can be seen when using the 685/730 nm band ratio.

This model was also used along with leaf fluorescence signatures to illustrate the fluorescent canopy variation between a normal and drought stressed soybean plant. It was shown that by using the ratio 685/730 nm the difference between the two canopies could be seen (see Figure 17) [Methy et al., 1994].

**Figure 17: Simulation of F_{690}/F_{730} Ratio from a Stressed and Unstressed Soybean**

![Graph showing simulation of the K_c ratio from a stressed (20 August 1990) and unstressed (4 July 1990) soybean crop canopy as a function of leaf area index: (-) planophile canopy: (- - -) erectophile canopy.

Both models by Rosema et al. (1991) and Olioso et al. (1992) show that LAI, canopy structure, and background reflection all affect the canopy fluorescence. Olioso does so by using a simpler model based on Beer-Lambert analytical equations while Rosema is based on the more intensive Kubelka-Munk differential equations. This is one reason why the model by Olioso was chosen. It allows more efficient calculation of canopy fluorescence.
Another reason for the decision is the model has been successfully utilized in calculating the ratio 690 nm/730 nm for varying plant conditions. Furthermore it has been used to illustrate that plant conditions can be discerned by measuring fluorescence at the canopy level.

So far the information presented has shown how several factors affect different aspects of the fluorescent signal. In this research the main interest is in how these factors affect the fluorescent ratio 690/730 nm. The following table (Table 3) summarizes how the different factors affect the fluorescent intensity and the ratio 690/730nm at the leaf and canopy (where applicable) level. This information was used to help design the specific scenario used in this research.

<table>
<thead>
<tr>
<th>Table 3: Factors Affecting Fluorescent Intensity and the Ratio 690/730 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor</td>
</tr>
<tr>
<td>Chlorophyll Concentration (Increases)</td>
</tr>
<tr>
<td>Leaf Side (Top vs. Bottom)</td>
</tr>
<tr>
<td>Sun vs. Shade Grown Leaf</td>
</tr>
<tr>
<td>Species Variation</td>
</tr>
<tr>
<td>Inhibited Photosynthetic Process (ex. Incr. DCMU)</td>
</tr>
<tr>
<td>Prolonged Dark Exposure</td>
</tr>
<tr>
<td>Water Stress (Increase)</td>
</tr>
<tr>
<td>Temp. Long Exposure</td>
</tr>
<tr>
<td>Low Temperature</td>
</tr>
<tr>
<td>High Temperature</td>
</tr>
<tr>
<td>Temp. Brief Exposure</td>
</tr>
<tr>
<td>Factor</td>
</tr>
<tr>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>Mechanical Injury (Incr.)</td>
</tr>
<tr>
<td>Nitrogen (Deficient)</td>
</tr>
<tr>
<td>Nutrient (Excess) (Deficient)</td>
</tr>
<tr>
<td>Senescence</td>
</tr>
<tr>
<td>Soil Salinity (Increase)</td>
</tr>
<tr>
<td>High Ambient Light Levels</td>
</tr>
<tr>
<td>UV-B Radiation (Long Exposure)</td>
</tr>
<tr>
<td>Air Pollution (Increase)</td>
</tr>
<tr>
<td>Ozone (Long Exposure)</td>
</tr>
<tr>
<td>Acid Rain</td>
</tr>
<tr>
<td>Metals</td>
</tr>
<tr>
<td>Excitation Intensity (Incr.)</td>
</tr>
<tr>
<td>Excitation Wavelength (λ)</td>
</tr>
<tr>
<td>Ambient Light Level (Incr.)</td>
</tr>
<tr>
<td>Canopy Depth (Lower)</td>
</tr>
<tr>
<td>Canopy Type- Increasing</td>
</tr>
<tr>
<td>Interceptiation Coefficient</td>
</tr>
<tr>
<td>Soil Reflectivity</td>
</tr>
<tr>
<td>Leaf Area Index (Incr.)</td>
</tr>
</tbody>
</table>

Incr. = Increases, Decr. = Decreases, ~ = Warrants Further Investigation. Chlr. = Chlorophyll Concentration, NC = No Change. The terms “research” or “model” indicated whether the results were obtained through actual research or by modeling of conditions.

This table lists the general trends in plant fluorescence when subject to different factors. This table is based on past research. It must be remembered that some cases are more documented than others. Therefore this summary by no means indicates the
absolute trend for each factor. Many factors influence a plant’s fluorescent signal. Therefore different plant and collection conditions may cause different trends in the plant’s fluorescent response. For this reason special attention has to be paid to the situation under which fluorescent measurements are made. This is why a specific scenario had to be devised when modeling a fluorescent signal. The specific conditions affecting the fluorescent signal had to be accounted for. The above table was used to aid in that effort.
2.6 $\textit{THESIS OBJECTIVES}$

With all these factors affecting a fluorescent signal the issue raised is whether a stress signature can be seen in a given situation? Detecting the signal using a remote sensing platform is the current situation of interest. Typically the research done so far has been at the leaf level. The question has become how would these leaf level signatures appear when measured from a distance? It is the objective of this research to pursue the answer to this question. In order to do this a remote sensing scenario was devised that provides a specific situation for collecting a fluorescent spectrum. The fluorescent signal received in such a situation must then be calculated. In order to do this the research focus was on the following objectives:

1) Identify the Plant and Stress Under Observation  
2) Identify Scenario Under which Fluorescence will be Calculated  
3) Obtain Leaf Level Fluorescent Ratio Considering Scenario Variables  
4) Obtain Canopy Level Fluorescence from Leaf Level Measurements by Utilizing a Canopy Model  
5) Radiometrically Determine Effects of the Atmosphere and Sensor on the Fluorescence Signal  
6) Statistically Determine if Fluorescent Ratio Values for Different Plant Conditions can be Distinguished at a Remote Level

This information was analyzed to answer the following questions. Are stresses seen at the leaf level still present in the fluorescent signature at the canopy level? What effect does the remote collection have on the value of the fluorescent ratio? What are some of the large noise factors in the collection process? Is the signal to noise ratio high enough to allow the fluorescent signal to be detected? The first step in addressing these questions was to devise a scenario. The scenario was devised to take into account other factors and reduce the variability they add to the signal. This was done using
information from existing work in the field. The objective was to take information from research that has been conducted under similar circumstances. This in effect reduced influence from differing factors.
3. Procedure for the Calculation of Canopy Level Fluorescent Efficiency

The following illustrates the mathematical calculations used to determine the fluorescence from a canopy at a particular wavelength. The method is based on the canopy model developed by Albert Olioso [Olioso et al., 1992].

First the basis of the model will be described followed by the procedure used to determine the canopy level fluorescent efficiency.

3.1 Attenuation of Radiation Due to the Canopy

It is assumed that the attenuation of radiation inside the canopy (both exciting and fluorescent energy) follows Beer-Lambert’s law. The attenuation coefficient is a function of the canopy structure and leaf optical properties. Equation 1 indicates the relationship between radiation level and canopy depth. It illustrates the amount of radiation at a specific level inside the canopy. The amount of radiation reaching a certain canopy depth is dependent on the amount of leaf mass encountered. The more leaf mass encountered the more the light is attenuated. The variable “L” dictates the amount of leaf mass encountered. It represents the cumulative leaf area index from the top of the canopy to the canopy depth in question.

\[ L = \text{additive leaf area index from top of canopy to a certain canopy depth} \]
\[ I(L) = \text{the radiation level at a depth L inside the canopy} \]
\[ k(L) = \text{attenuation coefficient of the canopy} \]
Equation 1

\[ \frac{dI(L)}{dL} = -k(L) \times I(L) \]

3.2 Exciting Radiation Reaching a Canopy Depth

The next step is to find a relation that allows the radiation to be determined at a certain depth inside the canopy (given the radiation at the top of the canopy). The radiation reaching a certain depth is a function of the canopy attenuating light. Finding the reduced light intensity due to attenuation is accomplished by integrating of Equation 1. It provides the relationship exhibited in Equation 2.

\[ I_0 = \text{amount of exciting radiation at the top of the canopy} \]
\[ k = \text{attenuation coefficient} \]

Equation 2

\[ I(L) = I_0 \times \exp\left[-\int_0^L k_e(L')dL'\right] \]

The attenuation coefficient changes depending on whether one is considering the exciting energy entering the canopy or the emitted fluorescence leaving the canopy towards the sensor. Therefore, depending on which situation is under consideration the coefficient \( k_e \) (exciting radiation) or \( k_f \) (fluorescent radiation) is used.
3.3 Leaf Fluorescence Due to Exciting Radiation

Having determined the amount of radiation at a particular canopy depth, the next step is to find the amount of resulting fluorescence. This is determined by taking the product of the fluorescent efficiency of the plant, the amount of exciting irradiance, and the interception coefficient of the plant. This allows one to determine the amount of emitted fluorescence at a canopy depth L. Equation 3 illustrates this relationship.

\[ F_i(L) = \text{fluorescent efficiency (leaf) as function of canopy depth L} \]
\[ K(L) = \text{interception coefficient of the plant canopy as a function of depth L} \]

Equation 3

\[ dF_i(L) = [F_i(L) \times K(L) \times I(L)]dL \]

The variable \( K(L) \) is the ratio of irradiance that intersects the normal of a leaf surface to \( I(L) \) (the amount of irradiance normal to a horizontal plane at a depth L inside the canopy). The variable is dependent on the type of canopy under consideration (erectophile, spherical, uniform, and planophile). A set of “K” values for different canopy types is listed in Table 4 [Olioso et al., 1992].

| Table 4: Attenuation (\( k_e \) and \( k_r \)) and Interception (K) Coefficients for Different Canopy Structures at a Leaf Chlorophyll Content of 40 µg/cm² |
|---|---|---|---|---|---|
| | \( \lambda \) (nm) | Erectophile | Spherical | Uniform | Planophile |
| \( k_e \) | 632.8 | 0.42 | 0.50 | 0.63 | 0.83 |
| \( k_r \) | 690 | 0.37 | 0.43 | 0.53 | 0.66 |
| \( k_f \) | 730 | 0.14 | 0.16 | 0.19 | 0.23 |
| K | 0.43 | 0.51 | 0.64 | 0.85 |
Table 5: Attenuation (ke and kf) Coefficients for a Spherical Canopy with Different Leaf Chlorophyll Content

<table>
<thead>
<tr>
<th></th>
<th>λ (nm)</th>
<th>10 μg/cm²</th>
<th>20μg/cm²</th>
<th>40μg/cm²</th>
<th>60μg/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>ke</td>
<td>632.8</td>
<td>0.43</td>
<td>0.47</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>kf</td>
<td>690</td>
<td>0.34</td>
<td>0.4</td>
<td>0.43</td>
<td>0.44</td>
</tr>
<tr>
<td>kf'</td>
<td>730</td>
<td>0.12</td>
<td>0.15</td>
<td>0.16</td>
<td>0.18</td>
</tr>
</tbody>
</table>

The model by Olioso assumes the leaf fluorescence efficiency (F(I(L))) to be independent of the radiation [Olioso et al., 1992]. As mentioned in the background the intensity of the radiation does not severely affect the overall spectrum of the fluorescence [Lichtenthaler et al., 1988]. However, as seen in Figure 6 [Lichtenthaler et al., 1988] and Figure 7 [Chappelle et al., 1987] the fluorescence is not independent of excitation wavelength. For this reason the model will be utilized at a particular excitation wavelength.

3.4 Fluorescent Signal Reaching the Top of the Plant Canopy

The next step is to find the amount of fluorescence reaching the canopy top for a particular layer inside the canopy. Assuming leaves act as lambertian emitters of fluorescence, Equation 4 illustrates how to find the fluorescence for a certain canopy depth.

\[ k_e(L') = \text{attenuation of exciting radiation by the canopy} \]
\[ k_f(L') = \text{attenuation of the emitted fluorescence by the canopy} \]
\[ dF_f(L) = \text{fluorescence reaching the canopy top from canopy layer L} \]
Equation 4

\[ dF_i(L) = F_i(L) \times K(L) \times I_o \times \exp\left[-\int_0^L \left[k_e(L') + k_f(L')\right] dL' \right] dL \]

As mentioned in the background the attenuation coefficients for exciting and emitted radiation are dependent on the canopy structure and chlorophyll level. This is illustrated by looking at the values of “\(k_e\)” and “\(k_f\)” in Table 4 and Table 5 [Olioso et al., 1992]. Table 4 shows how the attenuation variables change for different canopy types and for the wavelength under consideration. Table 5 shows how the attenuation variables change with varying chlorophyll level for a particular canopy type. Again the change due to wavelength is also shown. Changing the canopy type and chlorophyll levels of the canopy under consideration changes which attenuation values should be utilized as input to the canopy model.

In Figure 18 the sources of canopy fluorescence are shown. The diagram shows the fluorescent signal from a layer “\(L\)” It illustrates that the exciting light is attenuated (\(k_e\)) until it reaches layer “\(L\)” Then the induced fluorescence is attenuated (\(k_f\)) until it reaches the top of the canopy. As one goes deeper into the canopy the cumulative leaf area index increases therefore so does the attenuation of the radiation. This research and the above formula assume that the collection is done at a nadir point of view. This means the exciting and fluorescent energy will follow the same path in the canopy. The diagram shows an off nadir excitation but illustrates the general factors affecting the canopy signal.
The different contributions to the signal emitted by the canopy in sensor direction. Dashed lines represent exciting radiation and solid lines represent emitted radiation. Abbreviations are defined in the text. [Olioso et al., 1992]

3.5 Determine the Fluorescence Reaching the Canopy Top from a Particular Canopy Layer

The above equation can be simplified if the attenuation and interception coefficients are considered to be constant. This is true if a couple of assumptions about the canopy are made. The assumptions being that the canopy is homogeneous and does not vary in leaf optical properties or leaf angle distribution. The equation is then simplified to the form shown in Equation 5.

Equation 5

\[ dF_1(L) = F_1(L) \times K \times I_0 \times e^{-(k_r + k_f) \times L} \, dL \]

This provides the signal reaching the top of the plant due to the fluorescence of the plant at a certain canopy depth. The source of this fluorescence is illustrated in the left diagram in Figure 18.
3.6 Amount of Signal Exiting the Canopy from Fluorescence Reflecting from the Soil

The fluorescence from a plant layer not only emit towards the sensor but it also is emitted towards the soil. Therefore some of the signal received by a sensor is from energy being emitted by the plant and reflected from the soil towards the sensor. This is determined by modifying Equation 5 to include a soil reflectance term and the canopy density term (LAI, leaf area index). The resulting equation is Equation 6.

\[ \rho = \text{soil reflectance} \]
\[ \text{LAI} = \text{total leaf area index of the plant canopy} \]

**Equation 6**

\[ dF_2(L) = \rho \times F_1(L) \times K \times I_0 \times e^{-(k_e-k_f) \times L - 2 \times k_f \times \text{LAI}} \text{ dL} \]

This provides the amount of signal due to fluorescence reflecting off the soil and reaching the top of the canopy.

The second diagram in Figure 18 shows the factors affecting the above equation. The canopy depth affects the attenuation of the exciting and emitting light. The soil reflectivity dictates how much of the fluorescent energy is sent back towards the top of the canopy. Equation 6 adjusts which attenuation coefficients (exciting or fluorescent radiation) to use in the exponential term. Fluorescent energy from the canopy top emitted towards the soil and then reflected back traverses the canopy twice. The attenuation of the fluorescent energy is represented as "2k_e". As the exciting energy probes deeper in the canopy the "2k_e" term is reduced by "k_e x L". The amount of the reduction depends on the layer "L" reached by the exciting energy. The reduced fluorescent attenuation term is replaced with the exciting attenuation term "k_e". The
exponential term basically accounts for which attenuation factor to use based on the canopy depth in question.

3.7 Amount of Fluorescent Signal Exiting the Canopy Produced from Exciting Radiation Reflecting Off the Soil

Another portion of the fluorescent signal reaching the top of the canopy comes from the excitation energy reflecting off the soil and then inducing a fluorescent signal. This can been seen in the right diagram in Figure 18. What occurs is some of the laser energy, that is not intercepted by the plant canopy, is reflected off the soil. This reflected energy then induces fluorescence in the plant. The amount of canopy fluorescence due to this is found by modifying the attenuation terms found in Equation 6. In this case the exciting energy is assumed to traverse the entire canopy twice before inducing fluorescence at the very top of the canopy. This is represented by the term “$2k_e$” in the exponential term. In similar fashion to Section 3.6 the attenuation coefficient “$2k_e$” is partially replaced with the term “$k_f$” depending on how far into the canopy the fluorescence originates. The resulting equation is illustrated in Equation 7.

Equation 7

$$dF_3(L) = \rho \times F_i(L) \times K \times I_0 \times e^{-(k_f-k_e) \cdot L-2 \cdot k_e \cdot Ld} \, dL$$

This provides the amount of fluorescent signal exiting the canopy top due to excitation energy reflecting off the soil and inducing fluorescence. This is the last term used in determining the paths of canopy fluorescence.
3.8 Overall Fluorescent Signal Leaving the Canopy

The canopy fluorescence signal from a particular layer inside the canopy is found by adding together Equation 5 through Equation 7. In order to obtain the overall signal from every layer in the canopy this summation is integrated over all the layers of the canopy. The integration is integrated up to the total leaf area index of the plant. This relationship is illustrated in Equation 8.

\[ L = \text{additive leaf area index of canopy to a certain canopy depth} \]
\[ dF_1(L) = \text{the emitted fluorescence by a layer dL at depth L} \]
\[ dF_2(L) = \text{the reflection, in the sensor direction, of downward emitted fluorescence from the background (soil)} \]
\[ dF_3(L) = \text{fluorescence, in the sensor direction, from excitation energy reflected off the background (soil)} \]

**Equation 8**

\[ F_c = \int_0^L [dF_1(L) + dF_2(L) + dF_3(L)] \]

3.9 Canopy Depth Affects on the Fluorescent Efficiency at Bands 690nm and 730nm

Up to this point the fluorescent signal reaching a sensor was considered known for a particular fluorescent efficiency. However the efficiency changes due to canopy depth and changing chlorophyll levels in the leaves (see background discussion). This changing efficiency is different for bands 690 nm and 730 nm. As seen in Figure 19 the fluorescent intensity at 690 nm increases more rapidly with lower canopy depths. The
period and plus signs in the plot indicate actual experimental points of plant fluorescence at varying canopy depth. The dotted curves illustrate how these trends can be approximated with Equation 9 [Olioso et al., 1992]. By adjusting the variables “a”, “b”, and “c” one can dictate the general shape of the curves at 690 nm and 730 nm. The equation fitting the intensity data was used to approximate the how a leaf’s fluorescent efficiency changes with canopy depth.

\[
a = \text{percentage of fluorescence efficiency maintaining a mean output}
\]
\[
b = \text{percentage of fluorescent efficiency influenced by lower canopy depths}
\]
\[
c = \text{parameter adjusting the severity of changing fluorescent efficiency with canopy depth (adjusts exponential curve)}
\]
\[
L = \text{additive leaf area index with canopy depth}
\]

**Equation 9**

\[
F_{ij}(L) = a + b \times e^{cL}
\]

In order to properly characterize the difference between 690 nm and 730 nm different values for “c” are used for each waveband. This allows one to approximate the true change in fluorescent efficiency for each waveband in question.
Leaf fluorescence profiles following Eq. (9): (-) \( a = 0.94, b = 0.06, c = 0 \); (---) \( a = 0.94, b = 0.06, c = 0.7 \); (- -) \( a = 1.36, b = 0.20, c = 0.20 \); experimental points from 690 nm (o) and 730 nm (+) profile D in Figure 2. (see figure reference in paper by Olioso) [Olioso et al., 1992]

### 3.10 Adjustment of Process to Fit Procedure Used in the Final Calculations

The above equations illustrate the reasoning and calculations necessary to obtain the fluorescent signal for a plant canopy. The method, however, is for the signal at a particular excitation intensity \( I_0 \). The objectives of this research dictate the use of several different levels of excitation intensity. For this reason it was desired to have a canopy model that did not depend on radiometric calculations for laser intensity. Instead this term was removed from the above procedure. This allows the above model to be used in determining the fluorescent efficiency of a plant at the canopy level. Then different excitation intensities can be applied to determine fluorescent signal levels leaving the canopy. The radiometric process used in determining the excitation levels is discussed in the radiometric procedure section. The following set of equations was therefore used to determine the fluorescent efficiency of a plant at the canopy level.
The main equations used in modeling the canopy are as follows: Equation 9 is first used to determine the fluorescent efficiency at the leaf level for varying canopy depth. Then Equation 13 is used to integrate the following set of equations (Equation 10 through Equation 12). These formulas are obtained from dividing out the "I_0" term from Equation 5 through Equation 8.

**Equation 10**

\[
\varepsilon_1(L) = \frac{dF_1(L)}{I_0} = F_1(L) \times K \times e^{-(k_s+k_f)\times L} \, dL
\]

**Equation 11**

\[
\varepsilon_2(L) = \frac{dF_2(L)}{I_0} = \rho \times F_1(L) \times K \times e^{-(k_f-k_s)\times L-2\cdot k_s\times LAI} \, dL
\]

**Equation 12**

\[
\varepsilon_3(L) = \frac{dF_3(L)}{I_0} = \rho \times F_1(L) \times K \times e^{-(k_f-k_s)\times L-2\cdot k_s\times LAI} \, dL
\]

These equations are then utilized in Equation 13 to determine the overall fluorescent efficiency from the canopy. The integration was completed for a range of different LAI for the canopy.

**Equation 13**

\[
f\varepsilon(LAI) = \frac{F_\varepsilon}{I_0} = \int_0^{LAi} [\varepsilon_1(L) + \varepsilon_2(L) + \varepsilon_3(L)]
\]
By utilizing Equation 9 through Equation 13 the fluorescent efficiency of an entire plant canopy can be determined.
4. Procedure for the Radiometric Calculation of Remotely Detected Laser Induced Fluorescence of Vegetation

The following illustrates the mathematical calculations used to determine the fluorescent ratio (690 nm/730 nm) at a certain sensor platform. It includes the radiometric calculations and statistical analysis necessary to determine if two canopies, of different plant health, can be distinguished.

As a first step the amount of excitation energy reaching the plant needs to be calculated.

4.1 Divergence of the Laser Illumination

The divergence of the illumination is due to the laser and optical components involved in illuminating the target. In this case the divergence of the system is found by taking the tangent of the spot size diameter on the target divided by the height of the platform. The spot size diameter chosen was used to make sure a certain amount of target coverage is present at the canopy. It is assumed that the divergence of the laser system can be adjusted to the create the desired spot size by using the appropriate lens. In each platform system different collection optics were used in the design. This causes each system to have a different field of view. Therefore each platform needs to utilize a different divergence in it’s excitation beam. As seen in Figure 20 the illuminating system emits a beam with a certain angular divergence ($\theta$). This angular divergence can be determined if the height of the platform ($H$) and diameter ($D$) of the spot size (on the target) is known [see Equation 14]. In this case it is assumed that the divergence is a small enough angle where the tangent of “$D/H$” provides a reasonable value for “$\theta$“. In order to find the divergence of the illuminating system in steradians the area of the spot size on the ground needs to be determined. The area is then divided by the square of the illuminating distance [see Equation 15 and Equation 16]. In Equation 17 the final form
is shown where the beam divergence (in radians) is used to find the beam divergence in steradians ($\Omega$).

Divergence = $\theta$

Height above Target = H

Diameter of Beam Across Target = D

Divergence of Laser in Steradians = $\Omega_{\text{laser}}$

Radius of Illuminated Area = $r$

Area of Target Illuminated = A

Equation 14

$$\tan(\theta) = \frac{D}{H}$$

Equation 15

$$D = \tan(\theta) \times H$$

Equation 16

$$\Omega_{\text{laser}} = \frac{A}{H^2} = \frac{\pi \times (D/2)^2}{H^2}$$

Equation 17

$$\Omega_{\text{laser}} = \frac{\pi \times \left(\frac{\tan(\theta) \times H}{2}\right)^2}{H^2} = \frac{\pi \times \tan^2(\theta)}{4}$$
Figure 20: Diagram of the Factors Influencing Laser Irradiance

Radiance Intensity of a Laser Source
Determined by:
1) Energy per Pulse = Q (joules)
2) Time of Pulse = dT (seconds)
3) Divergence of Laser Beam (in Steradians)

Height of Sensor from Target = H

Diameter of Beam Across Target = D

Projected Diagonals of Various Platform Detectors
θ is Different for Each Platform
1) Portable Hand Held (10 m standoff) = 1.9648 m
2) Truck Based (30 m standoff) = 4.47 m
3) Airbased (300 m standoff) = 13.44 m

Irradiance Reaching Sensor = \( E_{\text{LASER}} \)
Determined by:
1) Height of Laser from Target = H
2) Transmission of Atmosphere at Laser Excitation Wavelength
4.2 Radiant Intensity of the Laser Source

In order to determine the radiant intensity of the laser it’s radiant flux (or power) must be determined first. This is done by dividing the laser energy per pulse (joules) by the pulse duration (seconds). This gives the number of watts per laser pulse. As can be seen in Figure 20 the divergence of the beam reduces the intensity of the radiant flux by spreading the power of the laser out over an area. Therefore, the radiant intensity is found by dividing the radiant flux of the laser by divergence (steradians) of the laser beam. This results in Equation 18 providing the radiant intensity.

Energy per Pulse [Joules] = Q
Pulse Time [sec] = dT
Divergence of Laser [steradians] = \( \Omega_{\text{laser}} \)
Intensity of Laser [watts/steradian] = \( I_{\text{laser}} \)

Equation 18

\[
I_{\text{laser}} = \frac{Q}{dT \times \Omega_{\text{laser}}} \quad \text{[watts/steradian]}
\]

4.3 Irradiance of the Laser Source Onto the Target

The distance of the laser source from the target along with the transmission of the atmosphere (at the wavelength of excitation) also affect the flux level reaching the target. These factors are taken into account when determining the irradiance on the target. Dividing the radiant intensity by the square of the laser distance provides the power per unit area of laser light reaching the target. The further the laser source is from the plant
the larger the illuminated area of the target (see Figure 20). Also the further away the source the more atmosphere the beam needs to pass through.

The atmospheric transmission is wavelength dependent and takes into account the loss of radiant intensity due to the atmosphere. The transmission in this case is determined for the wavelength at which the laser source is emitting. The atmospheric transmission at the excitation and fluorescent emission wavelengths is determined using the Lowtran software program. Lowtran models many attributes of an atmosphere given certain input parameters. Atmospheric transmission at the wavelengths of fluorescent emission is taken into consideration in the later steps. However the Lowtran model was not designed to provide accurate atmospheric transmission effects for a one nanometer bandpass. It assumes the information will be used over a larger bandpass region. Therefore when using the atmospheric transmission (from Lowtran) at the laser wavelength it is assumed that the value provided is accurate. An atmospheric model with enhanced spectral detail could improve the results.

Equation 19 illustrates how the two factors mentioned above are incorporated to provide the laser irradiance.

Height Above Target = H
Transmission of Atmosphere at Wavelength of Excitation = \( \tau_{atm}^{laser\lambda} \).

Equation 19

\[
E_{laser} = \frac{I_{laser}}{H^2} \times \tau_{atm}^{laser\lambda} \quad [\text{watts/meter}^2]
\]
4.4 Number of Photons Irradiated Onto the Target

The number of photons present in the laser irradiance needs to be found. The reason is that the known plant fluorescent efficiency values provide the number of photons out per incident photon. Therefore the number of photons exciting the plant have to be determined. First the laser irradiance is multiplied by the area illuminated. This provides the radiant flux on the plant. This is then multiplied by the laser pulse time. This gives the amount of radiant energy reaching the plant. Then, using the laser excitation wavelength, this value is converted into the number of photons reaching the plant. Equation 20 shows the method used to determine the number of excitation photons.

Wavelength of Laser Excitation = $\lambda_{\text{laser}}$
Plank’s Constant = $h$
Speed of Light = $c$
Area of Spot Size = $A = \pi(D/2)^2$

Equation 20

$$\text{Photons}_{\text{laser}} = \frac{E_{\text{laser}} \times \pi \times \left(\frac{D}{2}\right)^2 \times dT \times \lambda_{\text{laser}}}{\hbar \times c} \quad [\text{Photons}]$$

4.5 Number of Fluorescent Photons Emitted from the Plant

Once the laser irradiance reaches the plant it is both absorbed and allowed to pass through the plant. Some of the energy that is absorbed is remitted at a longer wavelength in the form of fluorescence. This is the signal that is desired for measurement with the detector. This signal has three components as seen in Figure 18 and Figure 21. The first
is the laser excitation that passes through the canopy, is reflected off the soil, and induces fluorescence on it's way back through the plant. The second is the fluorescence from the plant that is emitted towards the sensor. The third is the fluorescent energy that is emitted towards the soil and is reflected back through the canopy towards the sensor. The relative contribution of each amount is dependent on the fluorescence efficiency of the plant material (which is wavelength dependent), the soil reflectivity, canopy structure, and the leaf area index of the plant. The efficiency values taken for a plant material need to be approximated for a plant canopy. This is done by using a canopy model that incorporates the above factors to calculate the effects of the canopy on the leaf level fluorescent efficiencies. The model results provide the fluorescence efficiency of the plant canopy (number of photons out per incident photons). The canopy fluorescence efficiency is dependent on the leaf area index and the wavelength of emission (due to the characteristics of the fluorescent efficiency of the plant material).

Knowing the number of photons reaching the canopy the number of fluorescent photons can be determined. This is done by multiplying the number of exciting photons by the canopy fluorescent efficiency. The result, as seen in Equation 21, is dependent on the emission wavelength and leaf area index of the plant. It is assumed that the number of exciting photons is only due to the laser excitation source. Therefore it is assumed there is no solar induced fluorescence in the canopy’s fluorescent signal.

Number of Fluorescent Photons Emitted = FluorPhotons_\lambda(LAI)
Leaf Area Index of a Plant = LAI
Fluorescent Efficiency of a Plant Canopy(based on wavelength and LAI) = fe_\lambda(LAI)
(Determined from Canopy Model)
Equation 21

\[ FluorPhotons_\lambda (LAI) = Photons_{\text{laser}} \times fe_\lambda (LAI) \] [Photons]
Figure 21: Diagram of Factors Involved in the Excitation and Collection of Laser Induced Fluorescence

Fluorescence Radiance Signal \( \left( L_{\text{RADIANCE}} \right) \) from Plant Is Comprised of:

1) Ground Reflected Laser Excitation Inducing Plant Fluorescence
2) Fluorescence Emitted towards Sensor
3) Fluorescence Reflected off Ground towards Sensor

Depends on:
A) Irradiance on Plant from Laser = \( E_{\text{LASER}} \)
B) Fluorescence Efficiency of the Plant = \( f_e(\lambda, \text{LAI}) \)
C) Reflectivity of the Soil = \( \rho \)
D) Leaf Area Index of the Plant = \( \text{LAI} \)
4.6 Radiant Excitance of Fluorescent Energy from the Plant

Once the number of fluorescent photons is known it is desired to determine the plant's radiant excitance of fluorescent energy. In order to do this the number of photons is divided by the area of the spot size inducing fluorescence. It is also divided by the gatetime of the sensor system. This provides the time and area over which the fluorescent photons are collected. The number of photons is then converted to radiant energy in order to determine the fluorescent radiant excitance of the plant. The method is illustrated by Equation 22. The resulting radiant excitance values are wavelength and plant leaf area index dependent.

Radiant Exitance of Fluorescent Energy (based on wavelength and LAI) \( = M_{\lambda}(\text{LAI}) \)
Collection Period (Camera Exposure) = gate
Wavelength of Fluorescence \( = \lambda_{\text{fluorescence}} \)

Equation 22

\[
M_{\lambda}(\text{LAI}) = \left[ \frac{\text{FluorPhotons}_{\lambda}(\text{LAI})}{\text{gate} \times \pi \times \left( \frac{D}{2} \right)^2} \right] \times \left( \frac{\hbar \times c}{\lambda_{\text{fluorescence}}} \right) \text{ [watts/meter}^2]\]

4.7 Radiance of Fluorescent Energy from the Plant

The fluorescent energy emitted from a plant is assumed to be lambertian. With this assumption the next step is to determine the amount of energy transferred with respect to steradians. Since it is a lambertian emitter the radiance of the plant (in steradians) is found by dividing the radiant excitance by \( \pi \). This provides the amount of radiance leaving the plant into the hemisphere above the plant. Equation 23 shows that
the radiance is wavelength and leaf area index dependent since the radiant excitation is bound by the same parameters.

Radiance of Fluorescent Energy (with respect to wavelength and LAI) = \( L_{\text{plant}}(\lambda, \text{LAI}) \)

**Equation 23**

\[
L_{\text{plant}}(\lambda, \text{LAI}) = \frac{M_\lambda(\text{LAI})}{\pi} \quad \text{[watts/meter}^2 \times \text{steradian]}
\]

### 4.8 Amount of Reflected and Upwelled Radiance

One of the problems in trying to detect fluorescent signals is the amount of background noise formed by solar and skylight. These radiance values mask the fluorescent information. The background signals come in two basic forms. One is downwelled radiance that is reflected back towards the sensor. The second is upwelled radiance coming from the atmosphere in the path from the target to the sensor. The upwelled and downwelled radiance values are some of the information Lowtran obtains when modeling an atmosphere. The radiance values provided by Lowtran include direct solar illumination, atmospheric scattering of solar light towards the target, and emission of radiance from the atmosphere towards the target. The radiance information due to atmospheric self emission is only relevant at longer wavelengths (infrared). Since this research deals only with the visible spectrum these results from Lowtran were left out of the background radiance calculations.

Figure 22 shows the radiance terms dealt with in the fluorescent signal. They are the downwelled radiance from direct solar illumination and atmospheric scattering of solar light towards the target. The scattered solar radiance is what makes up the skylight
portion of the downwelled radiance. The upwelled radiance comes from the solar light being scattered towards the sensor by the atmosphere in the target to sensor path. While Lowtran provides the amount of upwelled radiance towards the sensor, the amount of downwelled radiance reflected towards the sensor needs to be calculated. Both the upwelled and downwelled radiance are wavelength dependent.

As shown in Figure 21 the amount of downwelled radiance reaching the sensor depends on the reflectivity of the plant. The reflectivity depends on the plant and the wavelength. For this reason the spectral reflectivity of a plant needs to be obtained. One way is to use a canopy reflectance model. In this paper the spectral reflectance was obtained by inputting the appropriate parameters into the PROSPECT reflectance model [Jacquemoud et. al, 1990].

The sum of the solar and sky irradiance is multiplied by the plant spectral reflectivity and the atmospheric transmission. This value is then divided by $\pi$ to give the amount of reflected radiance in the sensor direction (see Equation 26). The atmospheric transmission is included to determine how much of the reflected radiance reaches the sensor. As mentioned earlier the atmospheric transmission is also determined using Lowtran. The resulting reflected radiance value is wavelength dependent.

The upwelled radiance term is also wavelength dependent and the effects of atmospheric transmission are incorporated in the Lowtran calculation.

Equation 25 and Equation 26 provide the non-fluorescent background radiance reaching the sensor.

Before utilizing these equations the solar radiance reaching the ground had to be determined. Lowtran provides the exoatmosphere radiance from the sun and the atmospheric transmission to the ground. These terms had to be multiplied to find the solar irradiance actually reaching the ground. This is shown in Equation 24.
Irradiance from the Sun at the Exoatmosphere = \( E_{\text{sun}} \)

Transmission of Atmosphere from Exoatmosphere to the Ground = \( \tau_{\text{atm}} \)

Irradiance from the Sun reaching the Ground = \( E_{\text{sun,ground}} \)

**Equation 24**

\[
E_{\text{sun,ground}} = E_{\text{sun}} \times \tau_{\text{atm}}
\]

Irradiance from the Sky = \( E_{\text{sky}} \)

Transmission of the Atmosphere = \( \tau_{\text{atm}} \)

Reflectivity of the Target = \( r \)

Upwelled Radiance = \( L_u \)

Reflected Downwelled Radiance = \( L_{\text{reflected}} \)

**Equation 25**

\[
L_u = \text{from\_Lowtran} \quad \text{[watts/meter}^2\text{ * steradian]}
\]

**Equation 26**

\[
L_{\text{reflected}} = \frac{E_{\text{sun,ground}} \times \rho \times \tau_{\text{atm}} \times \cos \sigma}{\pi} + \frac{E_{\text{sky}} \times \rho \times \tau_{\text{atm}}}{\pi}
\]

The \( \cos \sigma \) term is needed to account for indirect solar irradiance. The further away from nadir the sun illuminates a horizontal surface the lower the amount of reflected irradiance. Since the average leaf inclination angle of a canopy can change (depends on canopy type) and affect this term, a worst case scenario was chosen. The
illumination angle \( \sigma \) was assumed to be zero. In doing this the reflected solar irradiance should not be higher for any canopy type being measured.
Figure 22: Diagram of Radiance Sources Influencing the Fluorescent Signal

Radiance Reaching Sensor
5) Laser Induced Fluorescence ($L_{PLANT}$)
6) Solar Reflected by Target
7) Skylight Reflected by Target
8) Upwelled Radiance from Atmosphere

1) Irradiance from Laser Excitation ($E_{LASER}$)
2) Irradiance from Direct Solar Illumination ($E_{SUN}$)
3) Irradiance from Scattered Solar reaching Target ($E_{SKY}$)
4) Irradiance from Scattered Solar towards Sensor
5) Radiance from Laser Induced Fluorescence Energy of Plant ($L_{PLANT}$)
6) Direct Solar Illumination Reflected by the Target ($L_{REFLECTED}$)
7) Scattered Solar Reflected by the Sensor ($L_{REFLECTED}$)
8) Scattered Solar towards Sensor ($L_{U}$)
4.9 Amount of Radiance Reaching the Sensor

Now that the radiant fluorescent and background signals are determined the total signal reaching the sensor can be found. This is done by adding together all the radiance signals reaching the sensor (see Figure 22).

First the effect the atmospheric transmission, from the plant to the sensor, has on the fluorescent radiance leaving the plant needs to be taken into account (it is already incorporated into the upwelled and reflected radiance terms in section 4.8). Once the product of the plant radiance and atmospheric transmission is taken, the upwelled and reflected radiance values are added to the product (see Equation 27). All these values are wavelength dependent and are added together at corresponding wavelengths.

Radiance Reaching the Sensor = L_{sensor}(LAI)

Equation 27

\[ L_{sensor}(LAI) = L_{plant}(LAI) \times \tau_{atm} + L_{u} + L_{reflected} \]

\[ [\text{watts/meter}^2 \times \text{steradian}] \]

4.10 F# of Detection System

With the radiance reaching the sensor known it is desired to find the irradiance on the detector. The value of radiance describes flux with respect to area and cone angle for a particular direction. Therefore the amount of area the sensor system maintains in the hemisphere above the target needs to be determined. This is done by finding the F# and subsequently the G# of the system. The F# is found from the focal length of the optics and the diameter of the entrance aperture of the system (see Equation 28).
4.11 Determine G# of the Detection System

When the radiance reaching the sensor is known the characteristics of the sensor, influencing the signal, must be taken into account. This is done by finding the G# of the collection system. It is found from incorporating the F# and the transmission characteristics of the system. The F# provides information on the collection area size of the system.

The transmission values come from any item in the sensor package that may attenuate the incoming signal. Therefore the transmission effects of items such as filters and lenses need to be taken into account. The transmission effects of each item are multiplied to form the transmission of the sensor ($\tau_{s}\text{sensor}$). Since these values are wavelength dependent so is the value of the G#.

Equation 29 shows the relationship in determining the G# of the system. One thing to keep in mind is the target needs to be functionally at infinity with respect to the collection optics or corrections to this equation need to be implemented.

Transmission of the Lens = $\tau_{l}\text{lens}_\lambda$
Transmission of the Filter = $\tau_{f}\text{filter}_\lambda$
Transmission of the Sensor System = $\tau_{s}\text{sensor}_\lambda = \tau_{l}\text{lens}_\lambda * \tau_{f}\text{filter}_\lambda$
Equation 29

\[ G_{\text{\#}} = \frac{4 \times (F\#)^2 + 1}{\pi \times \tau_{\text{sensor}}} \]

4.12 Irradiance on the Detector

Knowing the G\# provides information on the sensor characteristics; the entrance size and transmission of the collection system. Therefore dividing the radiance reaching the sensor by the G\# of the system will provide the amount of irradiance actually reaching the detector of the system (see Equation 30). The irradiance on the sensor is wavelength dependent.

Irradiance on the Detector = \( \text{Edetector}_{\lambda} (\text{LAI}) \)

Equation 30

\[ \text{Edetector}_{\lambda} (\text{LAI}) = \frac{L_{\text{sensor}}_{\lambda} (\text{LAI})}{G_{\text{\#}}_{\lambda}} \quad \text{[watts/meter}^2\text{]} \]

4.13 Radiant Flux on the Detector

The irradiance is the amount of flux per unit area reaching the detector. In order to find the response of the detector to the irradiance the amount of flux reaching each detector resolution element needs to be determined. This is done by multiplying the detector irradiance by the size of one resolution element (see Equation 31). This provides the radiant flux, which is wavelength dependent, at a resolution element.
Radiant Flux on a Detector Resolution Element = $\Phi_{\text{detector}}_{\lambda}$
Area of Each Detector Resolution Element = $A_{\text{resolution element}}$

**Equation 31**

$$\Phi_{\text{detector}}_{\lambda}(\text{LAI}) = E_{\text{detector}}_{\lambda}(\text{LAI}) \times A_{\text{resolution element}} \quad \text{[watts]}$$

### 4.14 Conversion of Radiant Flux from Watts to Photons

Sometimes the detector spectral response is given in relation to the number of incident photons on the detector (which is true for this research). In this case the radiant flux reaching the detector needs to be changed from watts to the number of incident photons. This is done by finding out how long (gate) the detector is exposed to the radiant flux ($\Phi_{\text{detector}}$). The product of these two values gives the number of joules incident upon the detector. This value can then be altered into photons for each wavelength in question. Equation 32 illustrates the steps needed to make such a conversion. The conversion and thus the output number of photons is wavelength dependent.

Number of Photons = $\text{photons}_{\lambda}(\text{LAI})$
Plank’s Constant = $h$
Time Exposure of Camera = gate
Speed of Light = $c$

**Equation 32**

$$\text{photons}_{\lambda}(\text{LAI}) = \frac{\Phi_{\text{detector}}_{\lambda}(\text{LAI}) \times \lambda \times \text{gate}}{h \times c}$$
4.15 **Signal Output from the Detector**

The next step is to determine the detector's response to the number of photons reaching the detector. The response of the detector depends on the incoming wavelength and the number of photons at that wavelength. Therefore the spectral response of the detector needs to be known. The sensor output is determined by integrating the product of the detector response and radiant flux over the wavelengths of interest (see Equation 33). The signal response determined this way is for one resolution element of the detector.

Quantum Efficiency of the Detector = QE_{detector} \lambda  
Signal Output from the Detector = Signal(LAI)  

**Equation 33**

\[
Signal(LAI) = \int [QE_{detector} \lambda \times photons_{\lambda}(LAI)] d\lambda
\]

4.16 **Signal to Noise Ratio of the Detector**

The signal to noise ratio (SNR) is necessary to determine if there is enough signal present to be detected over the noise. The characteristics of the detector influence how much noise is present in the output signal. Therefore in order to obtain a reasonable SNR value the characteristics of the camera need to be measured.

The camera system model in this research was a Xybion camera. This camera is currently used by Special Technology Laboratories in Santa Barbara, California to collect fluorescent imagery. This camera allows them to obtain images of a plant fluorescing. This group has conducted tests on this camera and they have determined the amount of output noise for a range of input signals (using typical camera gain settings). In order to
properly characterize the signal to noise ratio of the Xybion camera the relationship in Equation 34 was determined [Lutz, 1995]. It is assumed that the cameras in other systems would behave similarly.

In order to characterize the SNR the following items were considered. First of all the read noise encountered at each resolution element needed to be added to the signal before the square root is taken. The read noise is the information emitted by the camera when there is zero input to the system. The second variable added is Fcam. The Fcam value helps characterize how the system responds due to the gain settings used on the camera. This term is placed in the denominator and is multiplied by the square root term discussed above.

The result is a signal to noise ratio equation that is specific to the camera system modeled in this project (see Equation 34). The SNR is dependent on the leaf area index being observed.

Excess noise factor in camera = Fcam
Read noise from camera with zero input (for one resolution element) = ReadNoise

**Equation 34**

\[
SNR(LAI) = \frac{Signal(LAI)}{Fcam \times \sqrt{Signal(LAI) + ReadNoise}}
\]

### 4.17 Noise (Standard Deviation) in the Camera Signal

Once the signal to noise ratio has been obtained, the amount of camera noise produced from a certain strength signal can be determined. This is done by dividing the signal strength by the SNR. This provides the standard deviation from the mean
measured output from the camera. This standard deviation is the noise induced by the camera and it is also dependent on the leaf area index.

The term "n" in Equation 35 is present in case pixel averaging in necessary. Due to the low levels of fluorescent signal the noise inherent in the system may be too large to be able to assess plant condition. If this is the case pixel averaging may be necessary to lower the noise level in the system. This will increase the ability to discriminate between plant conditions (higher signal to noise) but at the loss of spatial resolution. By averaging over "n" resolution elements the noise is reduced by a factor of square root "n"

Standard Deviation of the Signal Due to Noise = \( \sigma(LAI) \)
Number of Resolution Elements Averaged = n

Equation 35

\[
\sigma(LAI) = \frac{\text{Signal}(LAI)}{\text{SNR}(LAI)} \cdot \frac{1}{\sqrt{n}}
\]

4.18 Radiometric Signals from the Wavebands 690 nm and 730 nm

The signal, SNR, and noise level need to be obtained for the bands of interest. In this research those bandwidths are around the wavelengths 690 nm and 730 nm.

Signal for Waveband 690 nm = Signal690(LAI)
Signal for Waveband 730 nm = Signal730(LAI)
Standard Deviation of the Signal at 690 nm and 730 nm = \( \sigma_{690}(LAI) \), \( \sigma_{730}(LAI) \)
4.19 Ratio of the Two Wavebands 690 nm and 730 nm

The reason for obtaining the signal at 690 nm and 730 nm is to generate a ratio of the two bands. In the past, this ratio (Index) has shown the ability to indicate plant condition (see background). The plant index is determined by dividing the signal at 690 nm by the signal at 730 nm across varying leaf area indexes (see Equation 36). Therefore the ratio is a function of leaf area index.

Ratio of the Camera Signal Output at the 2 Wavebands (690nm/ 730 nm) = Index(LAI)

Equation 36

\[
\text{Index(LAI)} = \frac{\text{Signal}_{690(LAI)}}{\text{Signal}_{730(LAI)}}
\]

4.20 Standard Deviation in the Index Value

Having obtained the index of a plant, the variation in the index due to camera noise needs to be determined. The variation in the index is a result of the noise present at 690 nm and 730 nm.

When two gaussian distributed functions are divided the resulting relationship of the output distribution is found in Equation 37. The result is a ratio of the output variance divided by the square of the output mean. This term is equal to the sum of the same ratio applied to the two original distributions, then subtracted by a covariance term.

The covariance term between distributions u and v is utilized when there is correlation between the standard deviations of the two distributions. When the two distributions are uncorrelated the term goes to zero and drops out of the equation. In this case to obtain the variance of the output, the remaining two terms on the right hand side
of the equation are multiplied by the square of the output mean. The square root of both sides of the equation are taken to obtain the standard deviation of the output mean (plant index).

In this research the variances in the signal are correlated. This is due to the fact they are both a result of the same source, camera noise. However it was assumed that there was no correlation present in the camera noise of bands 690 nm and 730 nm. This describes a worst case scenario and the last term in Equation 37 drops out as described above. This insures that the results should not be any worse under real collection conditions. Another reason for this decision was that the correlation between bands 690 nm and 730 nm was not known.

Equation 38 shows the resulting equation where the noise in plant index is obtained from the mean of the output index and the mean and noise of the signals at 690 nm and 730 nm. The relationship is dependent of the leaf area index of the plant.

Standard Deviation of Distributions u and v = \( \sigma_u, \sigma_v \)
Mean of Distributions u and v = \( \mu_u, \mu_v \)
Covariance between Distributions u and v = \( \sigma_{uv} \)
Mean of New Distribution x = \( \mu_x \)
Standard Deviation of New Distribution x = \( \sigma_x \)

**Equation 37**

\[
\frac{\sigma_x^2}{x^2} = \frac{\sigma_u^2}{\mu^2} + \frac{\sigma_v^2}{\mu^2} - 2 \times \frac{\sigma_{uv}^2}{\mu \times \nu} \quad \text{[Bevington, 1969]}
\]

Standard Deviation of the Index Value = \( \sigma_{\text{Index(LAI)}} \)
\[ \sigma_{\text{Index}(LAI)} = \sqrt{\left(\frac{(\sigma 690(LAI))^2}{(\text{Signal}690(LAI))^2} + \frac{(\sigma 730(LAI))^2}{(\text{Signal}730(LAI))^2}\right) \times (\text{Index}(LAI))^2} \]

### 4.21 Average Index Signal

The model used to generate the fluorescent efficiency of a plant canopy takes into account the amount the fluorescent signal changes with canopy depth, soil reflectivity, and leaf area index. The variation due to the leaf area index has been included but the change in fluorescence due to variation in soil reflectivity and amount of increase in fluorescence at lower canopy depths have yet to be included.

A region of interest may not consistently have the same soil reflectivity or canopy depth factors. Therefore the variability in these canopy values adds to the variability in the recorded fluorescent signal. These parameters influence the fluorescent signal and need to be accounted for in determining if changes in plant condition can be seen remotely.

This is done by creating several signals using different variables for soil reflectivity and canopy depth fluorescence. The variables chosen represent the extreme values the factor (soil reflectivity or canopy depth) may possess. This creates a range of possible signal levels (in this research four different canopy parameter sets were used). The canopy fluorescent efficiencies of this range of canopy conditions were determined. This set of canopy efficiencies was generated for use in Equation 21. The efficiencies were found by using the extreme variables mentioned above. This process provides multiple 690 nm and 730 nm signals for the plant. The net result is a set of four index values and standard deviations for a plant. In order to obtain the average of these indexes
they are added together and divided by the number in question (see Equation 39). The average index is leaf area index dependent.

Plant Index Values for Four Different Canopy Parameters = \( \text{Index}_a, \text{Index}_b, \text{Index}_c(\text{LAI}) \)

Average Value of the Index for all Four Canopy Parameters = \( \text{AverageIndex}(\text{LAI}) \)

**Equation 39**

\[
\text{AverageIndex}(\text{LAI}) = \frac{\text{Index}(\text{LAI}) + \text{Index}_a(\text{LAI}) + \text{Index}_b(\text{LAI}) + \text{Index}_c(\text{LAI})}{4}
\]

### 4.22 Standard Deviation in the Average Index

Once the average index of a plant is determined the amount of deviation in this term needs to be calculated. This is done by using the relationship in Equation 40. It provides the deviation in the input signal reaching the camera. This provides the variation in the plant index from the canopy itself (not the camera).

Standard Deviation of the Indexes Different Canopy Parameters from the Mean Canopy Index = \( \sigma \text{AverageIndex}(\text{LAI}) \)

**Equation 40**

\[
\sigma \text{AverageIndex}(\text{LAI}) = \sqrt{\frac{[\text{Index}(\text{LAI}) - \text{AverageIndex}(\text{LAI})]^2 + [\text{Index}_a(\text{LAI}) - \text{AverageIndex}(\text{LAI})]^2}{3} + [\text{Index}_b(\text{LAI}) - \text{AverageIndex}(\text{LAI})]^2 + [\text{Index}_c(\text{LAI}) - \text{AverageIndex}(\text{LAI})]^2}
\]
4.23 **Average of the Index Noise**

Each index that is added in the average has a standard deviation due to camera noise. At this point the mean signal (index), due to plant variances, is known. The mean noise from the camera for this mean index needs to be determined. In order to do this the noise from the camera, for each of the four canopy signals, is taken and averaged. This is the same method used to determine the average index value. This is done by adding the noise terms and dividing by the number added together (see Equation 41). The term is also leaf area index dependent. This provides the expected average fluorescent ratio from a certain plant condition along with the expected average variation in the ratio.

Standard Deviation in the Index Signal (due to the camera) of 4 Canopy Signals = \( \sigma_{\text{Index}\ldots a,b,c(LAI)} \)

Average Standard Deviation from All 4 of the Signal Indexes = Average\( \sigma_{\text{Index}}(\text{LAI}) \)

**Equation 41**

\[
\text{Average}_\sigma\text{Index}(\text{LAI}) = \frac{\sigma_{\text{Index}(\text{LAI})} + \sigma_{\text{Index}_a(\text{LAI})} + \sigma_{\text{Index}_b(\text{LAI})} + \sigma_{\text{Index}_c(\text{LAI})}}{4}
\]

4.24 **Overall Noise in the Index**

In order to determine the overall standard deviation in the mean index value, the noise due to signal variation and camera noise needs to be combined. This is done by adding the values in quadrature.

Therefore to determine the standard deviation in the index, the square root is taken of the sum of both input values squared. The input values are the average standard
deviation due to camera noise (from the four input signals) and the standard deviation due to the signal variation from the plant canopy. The resulting equation is also leaf area index dependent (see Equation 42).

Combined Standard Deviation in the Index Value = Combinedσ(LAI)

Equation 42

\[ Combinedσ (LAI) = \sqrt{(σAverageIndex(LAI))^2 + (AverageσIndex(LAI))^2} \]

4.25 Index and Standard Deviation for Both a Healthy and Stressed Plant

The above process needs to be implemented for two different situations. These situations being a healthy and stressed plant. By determining the index and standard deviation of a plant in these two conditions, an assessment can be made if enough separation is present in the signals to detect a difference.

Index of Healthy Plant = IndexHealthy(LAI)
Index of Stressed Plant = IndexStressed(LAI)
Standard Deviation of Healthy Index = σIndexHealthy(LAI)
Standard Deviation of Stressed Index = σIndexStressed(LAI)

4.26 Separation of the Indexes over the LAI

Once the index values of two plant conditions are calculated the difference between the two values can be found. This is done by subtracting the two index values over the leaf area index of the plant (see Equation 43).
Separation of the Indexes = IndexDifference(LAI)

**Equation 43**

\[ \text{IndexDifference}(LAI) = \text{IndexStressed}(LAI) - \text{IndexHealthy}(LAI) \]

**4.27 Minimum Difference Needed to Distinguish a Change in Index**

The final step is to determine the minimum separation needed to identify one plant index from another. This is done by utilizing Equation 44.

The minimum separation is determined by multiplying a z-score value by the square root value listed in Equation 44 [Gescheider, 1985]. A z-score value of 1.04 is used in the equation. This provides the minimum separation needed to distinguish between the two signals with a confidence interval of 85%. A different confidence level can be used by altering the z-score accordingly. The first two terms under the square root are the squares of the standard deviations of the two distributions under consideration. The third term is for when the two distributions are correlated. It is assumed that the healthy and stressed plant signals are uncorrelated. Therefore this third term reduces to zero. This assumption is not necessarily true. However as mentioned before by assuming uncorrelated results it takes into account a worst case scenario. The results then provide values applicable to a wider range of conditions.

The equation incorporating plant indices and standard deviations is listed in Equation 45. This illustrates how the minimum difference, needed for detectability, is found. It's value is a function of the leaf area index.

\[ \mu_A, \mu_B \]
\[ \sigma_A, \sigma_B \]
Correlation Coefficient between A and B = \( r_{\mu_A\mu_B} \)

\( Z \)-Score = \( Z_{BA} \)

**Equation 44**

\[
\mu_B - \mu_A = Z_{BA} \times \sqrt{\sigma_{\mu_A}^2 + \sigma_{\mu_B}^2 - 2 \times r_{\mu_A\mu_B} \times \sigma_{\mu_A} \times \sigma_{\mu_B}}
\]

Minimum Change in Index Needed to Detect a Change = \( \text{MinimumDifference}(\text{LAI}) \)

**Equation 45**

\[
\text{MinimumDifference}(\text{LAI}) = 1.04 \times \sqrt{(\sigma\text{IndexStressed}(\text{LAI}))^2 + (\sigma\text{IndexHealthy}(\text{LAI}))^2}
\]

\( Z \)-score used for 85% confidence interval was 1.04.

**4.28 Determine the Separability of Different Plant Conditions**

The last step is to determine if the plant conditions are separable. This is completed by comparing the separation of the indexes (found in step 4.26) with the minimum difference needed for separability (found in step 4.27). This is done over the range of leaf area indexes of the plant.
5. **Modeled Scenario**

The following is a presentation of the conditions used to model the fluorescent ratio (690 nm/730 nm) reaching a sensor. It is an overview of the specific scenario used for making the radiometric calculations. The reasoning and assumptions used in creating this theoretical environment are discussed in later sections. The scenario built in this research included several different collection situations.

The fluorescence signal was looked at for three different sensor platforms. The platforms were designed to collect fluorescence at the greenhouse, ground based (field crop), and airborne levels. The three different sensor situations were used to determine the fluorescent signal from soybean plants.

It was decided to calculate the fluorescent ratio (690 nm/730 nm) for six different soybean conditions. The conditions being four different levels of chlorophyll concentration and the soybean plant before and after a herbicide stress. The signals for all six conditions at all three sensor heights were found.

The signals were calculated for an afternoon collection. The scenario was also built to view the plant at nadir utilizing an excitation laser at 632.8 nm. The soybean canopy was assumed to be a spherical type canopy.

5.1 **Assumptions and Approach of Building the Scenario**

Having listed the procedures used to determine the canopy model and radiometric calculations the next step will be to show how they were implemented. This section will discuss the decisions and assumptions made in calculating sensor signals for a particular scenario. The information used to make these calculations will be illustrated. The first step was to decide upon the scenario assumptions under which the calculations would be made.
5.1.1 Time of Day (Ambient Light)

It was decided to model the measurements for a 5 P.M. collection time. The reason being the daylight is still intense enough for the plants to remain photosynthetically active. However, at that time of day the intensity is lower to allow for less background signal in the measured fluorescence.

The reason a particular time of day was chosen is the fluorescent signal varies with ambient light level (time of day). As mentioned in the background, the more intense the ambient light the lower the ratio 690 nm/730 nm. Past research has presented a qualitative trend with ambient light but it has not produced a quantitative one. It appears that the excitation wavelength, species type, and plant condition affects a plant's fluorescent response in varying ambient light levels. Being a relatively new area of research no specific values were available as to soybeans response to different times of day. Therefore a specific time of day was chosen to remove the variability due to changing ambient light levels.

When making such measurements under real conditions it becomes imperative to make the measurements at the same solar elevation. Otherwise the comparability of the measurements suffers. One way to increase the comparability of two separate collections would be to make an ambient light measurement prior to the collection of laser induced fluorescence from the plant.

5.1.2 Collection Point of View (Sensor Position)

In the background it was mentioned that leaf side (top or bottom) affects the fluorescent signal measured from the plant. The lower sides of leaves develop less chlorophyll and therefore fluoresce more when excited with a laser. When measuring the fluorescence of a canopy, viewing both tops and bottoms of leaves adds to the variability in the measured signal. In order to reduce this factor it is desirable to view only one side of the leaves.
This can be accomplished by using a nadir point of view when looking at the canopy. The top view of the canopy aids in measurement because less branch material is seen and fewer under sides of leaves are visible. This increases the SNR while providing less variability in the signal [Mentioned in Laboratory Discussion Group during the Fluorosensing Information Exchange at EPCOT Orlando, Florida 2/95] (may not be the case in higher wind conditions).

5.1.3 Excitation Wavelength

It was decided to model a scenario with excitation light at 632.8 nm. One of the main reasons for this was the input parameters given in the canopy model [Olioso et al., 1992] were for an excitation at 632.8 nm. Another reason is much of the research in the past was conducted at this wavelength. A reason for this is past laser technology made this type of laser readily available. Therefore this research models a situation many of the systems in the research field could emulate. This wavelength laser is also readily available at many different excitation powers. As laser technology grows, higher power and different wavelength lasers will be built. This will allow fluorescent research to be conducted at wavelengths other than the limited few utilized currently. The current technology limits what wavelengths can be realistically used when remotely exciting fluorescence in plants.

The problem with 632.8 nm is this is not the best wavelength to use for making fluorescent measurements in plants. Lower wavelengths (ex. 450 nm) are more efficient at exciting the chlorophyll of the plant. Also as one excites the plant past 525 nm different excitation wavelengths cause different fluorescent spectrum from the plant. Therefore it is suggested in future work to pursue a system that excites a plant at a lower wavelength of laser excitation. This should provide higher signal levels and more comparability with other experiments (conducted at lower excitation wavelengths).
5.1.4 Plant Type

The plant type used in this research is that of a soybean plant (*Glycine max*). The main reason for this choice was that the canopy model by Albert Olioso included input parameters for a soybean plant. Besides this there was also pertinent information on stress relating to a soybean plant. In fact Figure 16 shows that differences in chlorophyll content were distinguished using a canopy level fluorescent signal. The goal of this research was to take it one step further and see if the differences were still visible at a remote location.

Of the past research in plant fluorescence the two main types of plant studied were either cash crop plants or those occupying large areas of forests. Another reason soybean was chosen was due to its being a cash crop and having been the subject of fluorescent studies in the past.

5.1.5 Stress Conditions

There are two main reasons why stress will cause plants to change their fluorescent output. One reason is that stress can cause a change in the plant’s chlorophyll content. The other reason is stress can cause the photosynthetic process to be inhibited. It was desired to chose situations that would allow both stress types to be ascertained. This is why it was decided to consider the soybean plant under two different types of stress.

The first situation being the changing output of a soybean plant due to changing chlorophyll levels. It has been shown in the past that these changes can be seen at the canopy level (see Figure 16) [Olioso et al., 1992]. One reason this condition was chosen is many sources of stress lead to a breakdown or inadequate development of chlorophyll. Also the canopy model used contained the varying attenuation coefficients needed to properly model the changing chlorophyll conditions. These values are listed in Table 5 [Olioso et al., 1992].
Another reason for choosing this type of stress is the fluorescent ratio for each chlorophyll level is known (see Figure 23) [Olioso et. al, 1992].

**Figure 23: Leaf F$_{690}$/F$_{730}$ Ratio as a Function of Leaf Chlorophyll Content**

[Diagram showing a graph with chlorophyll content on the x-axis and F$_{690}$/F$_{730}$ ratio on the y-axis.]

Leaf F$_{690}$/F$_{730}$ ratio as a function of leaf chlorophyll content. Measurements on soybean leaves, Montpelier, Summer 1990. [Olioso et. al, 1992]

This plot shows that as the chlorophyll level increases the resulting ratio decreases. This plot was used to determine the fluorescent ratio of 690 nm/730 nm for chlorophyll levels 10, 20, 40 and 60 $\mu$g/cm$^2$. The resulting ratios are listed in Table 6.
Table 6: Ratio of Leaf Level Fluorescence (690 nm/730 nm) for a Soybean Plant

<table>
<thead>
<tr>
<th>Chlorophyll Concentration</th>
<th>Herbicide added to Soybean with 40 μg/cm² Chlorophyll</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll</td>
<td>Ratio</td>
</tr>
<tr>
<td>10 μg/cm²</td>
<td>1.35</td>
</tr>
<tr>
<td>20 μg/cm²</td>
<td>1.15</td>
</tr>
<tr>
<td>40 μg/cm²</td>
<td>0.8</td>
</tr>
<tr>
<td>60 μg/cm²</td>
<td>0.6</td>
</tr>
</tbody>
</table>

The second type of situation causing a change in fluorescence is inhibiting the photosynthetic process. One way this can occur is through herbicide stress. This is a problem because the very tool used to remove pests from a crop can inhibit the growth of that crop. One type of herbicide that is often studied is DCMU. Therefore it was decided to consider the fluorescent signal of a soybean plant undergoing a DCMU stress. One problem was the change in a soybean leaf's fluorescence signal due to DCMU stress was not found in the literature. Therefore the leaf level fluorescence was assumed to behave like that of a tobacco plant (Nicotiana tabacum). The change in ratio from a healthy to a DCMU stressed Nicotiana tabacum plant is listed in Table 2 [Lichtenthaler et al., 1998]. The ratio of a healthy plant (0.97) and DCMU stressed plant (1.18) used in this experiment are also listed above in Table 6.

5.1.6 Leaf Level Fluorescent Efficiency Values

Having determined what fluorescent ratios can be expected for different plant conditions the next step was to find out what the fluorescence efficiency of the plant is under these conditions. Unfortunately fluorescent efficiencies values were not available for a soybean plant in each of the above listed conditions. In fact the fluorescent
efficiency of any condition soybean plant was not known. It had to be assumed that soybean fluorescent efficiencies were similar to corn (maize-another crop plant). The fluorescent efficiency curve of the corn plant is shown in Figure 24.

**Figure 24: Fluorescent Efficiency of Corn (excited at 532 nm) versus Wavelength**

![Fluorescent Efficiency Curve](image)

[Data from Special Technology Laboratories, Santa Barbara, California]

Of course the fluorescence of plants change due to species type. Therefore in order to make an appropriate assumption a fluorescent output curve from a soybean plant was compared to the QE curve for maize. The scenario modeled in this research is utilizing a laser with excitation at 632.8 nm. The fluorescent output of a soybean plant excited at 632.8 nm can be seen in Figure 25.
Fluorescence emission spectrum excited by an UV laser (337 nm) and chlorophyll fluorescence emission spectrum excited by an He/Ne laser (632.8 nm) of a green leaf of soybean grown in the field. The UV-laser beam does not reach the mesophyll cells of the leaf of the field plant and therefore does not excite chlorophyll fluorescence.

This spectral curve was compared to fluorescence efficiency curves from four different types of plants (lawn grass, eucalyptus leaf, pine needles, and dry corn leaf). It was determined that the curve from a corn plant excited at 532 nm produced a curve that was close to a soybean plant excited at 632.8 nm. Figure 24 shows the fluorescent efficiency curve for a corn plant exited at 532 nm. By comparing this plot with that of the soybean the similarities are evident.

Mentioned earlier was the effect different excitation wavelengths can have on the output fluorescence, especially above 525 nm. By comparing the output curves it is intended to reduce the effect the different excitation wavelength may have had on the output. One assumption of note however is that the fluorescence efficiency of a soybean plant (excited at 632.8 nm) is similar to the fluorescent efficiency of a corn leaf (excited at 532 nm). This was the assumption used to obtain realistic fluorescent efficiency values for the bands 690 nm and 730 nm. Future work in the area should look to obtain quantum efficiency values for the same plant at the excitation wavelength of interest.
This is an important factor in improving the results of the calculations presented in this research. Ideally the quantum efficiency values should be determined for the plant under each different plant condition (healthy and stressed). In this study, to obtain fluorescent efficiency values for bands 690 nm and 730 nm their bandwidths had to be determined.

The intent of this research was to model the fluorescent signal of a soybean plant by an actual laser induced fluorescence system. The system under analysis utilized two separate filters to isolate fluorescence at 690 nm and 730 nm. These curves are shown in Figure 26.

**Figure 26: Filter Transmission Curves from Sensor Platforms**

![Filter Transmission Curves](image)

[Data from Special Technology Laboratories, Santa Barbara, California]
The bandwidth of these filters was considered to be the bandwidth of interest around the wavelengths 690 and 730 nm. The bandwidths were chosen as 661 nm to 699 nm (690 nm bandwidth) and 718 nm to 760 nm (730 nm bandwidth).

The quantum efficiency of the corn leaf with excitation at 532 nm was found to be 0.8% [Lutz, 1995]. This value was determined by the Special Technologies Laboratory in Santa Barbara, California. The value was termed the quantum efficiency of the plant. However, this is not the emitted fluorescence due to the number of absorbed photons. The value was determined as the amount of fluorescence due to the photons incident on the surface of the plant. This value was determined by integrating over the entire spectrum found in Figure 24.

In this research, the quantum efficiency was found in the following manner. The percent of quantum efficiency for a bandwidth (small or large) was determined by first adding all the fluorescent efficiency values (shown in Figure 24) over the wavelengths of interest. This was then divided that by the sum of fluorescent efficiency values over the whole spectrum. This is shown in Equation 46. The percent fluorescent efficiency at the waveband of interest was then multiplied by the total quantum efficiency of the plant from 300 to 800 nm. This provided the fluorescent efficiency for a particular wavelength interval.

Equation 46

\[
FE_{300}^{730} = \sum_{\lambda_3}^{\lambda_2} \frac{FE_{Plant}}{TotalQE} \times \sum_{300}^{800} FE_{Plant}
\]

By utilizing a small \( \Delta \lambda \) the spectral shape from Figure 24 is preserved. However, it was assumed that the fluorescent efficiency values over the region of interest (band 690 nm =
661 nm to 699 nm and 730 nm = 718 nm to 760 nm) do not vary rapidly. This assumption was made to allow the adjustment of the quantum efficiency values for different plant conditions. Therefore FE_{690,\Delta\lambda} and FE_{730,\Delta\lambda} was determined over the bandwidths of 690 nm and 730 nm.

However, many of the calculations being dealt with in the radiometric model are wavelength specific. The calculations were made at every 1 nm interval. In order to obtain the fluorescent efficiency value at every wavelength terms FE_{690} and FE_{730} were divided by the number of nanometers in the region. This is illustrated in Equation 47.

**Equation 47**

\[
FE_{per\_nm730} = \frac{FE_{730,\Delta\lambda}}{nm\_in\_Bandpass(\Delta\lambda)}
\]

This provides the quantum efficiency for every 1 nm interval in the 690 nm and 730 nm region. A drawback of this process is the resulting fluorescent efficiency values are the average over the waveband. The spectral information over the waveband is lost. A benefit is that the fluorescent efficiency values can now be easily altered to fit the stress condition under consideration.

The purpose of the previous steps was to obtain a realistic fluorescent efficiency for a soybean plant. The condition of the corn plant used to obtain the QE values is not known. The plots of soybean and corn fluorescence (see Figure 25 and Figure 24) only allow one to gain information on relative fluorescent efficiency for signal strength issues. The ratio of the bands 690 nm and 730 nm from Figure 24 turned out to be 0.32. Which is the correct ratio for the corn plant whose condition is not readily known. The condition of a plant has a direct impact on that plant’s fluorescent efficiency and therefore it’s ratio of the band 690 nm and 730 nm. The quantum efficiency of soybean
plant under different conditions was not known but the ratio of the bands 690 nm by 730 nm was known. The ratios of certain soybean conditions were used to calculate the quantum efficiency values at those ratios. This was done in the following fashion.

First the fluorescent efficiency at 730 nm was assumed to remain constant over different plant conditions. This was based on the fact that past research appeared to illustrate that the output at 690 nm varied more than that at 730 nm for different plant conditions. The fluorescent efficiency at 690 nm was then generated with respect to the fluorescent efficiency of 730 nm and the plant ratio under consideration. Equation 48 illustrates the calculation used to determine this.

**Equation 48**

\[
AdjustedFE_{690} = Ratio \times FE_{730}
\]

The fluorescence efficiency of 690 nm was determined for every ratio listed in Table 6. The results of all these calculations was to provide the fluorescent efficiency, at 690 nm and 730 nm, of a soybean plant under varying health conditions.

Having obtained the efficiencies at the leaf level the next step is to determine the canopy level efficiencies.

### 5.1.7 Canopy Density

In order to determine the canopy level efficiencies the density of the canopy has to be incorporated in the calculations. As shown in the background, by the canopy model, fluorescence is influenced by leaf area index (canopy density). The issue becomes what leaf area index values are appropriate to model a soybean canopy. It was decided to utilize a LAI range that went from zero to six. One reason for this decision was this range was previously used to generate soybean canopy data [Olioso et al., 1992]. It was also based on Figure 27. This plot shows the LAI for soybean during growing
seasons of two different years (1988, 1989). The plots indicate that after more than 35 growing days soybean plants have an LAI ranging from 2 to about 5.5. So as long as the plant is a month old it should have an LAI of at least two. For the cases where measurements of fluorescence may be made during early plant growth, a range of LAI from zero to six was utilized in modeling the canopy.

Figure 27: Leaf Area Index of Soybean Over the Growing Season

Seasonal leaf area index in Williams 82 soybean, based on treatment means over soil moisture levels, as influenced by inter-row spacing (0.36 and 1.02 m) and year. Vertical bars represent confidence intervals (α = 0.05) at selected points on leaf area index curves. [Savoy et al., 1992]

5.1.8 Canopy Fluorescent Efficiency

In order to determine the effects of the canopy on leaf level fluorescence a canopy model was used. The canopy model, discussed earlier, allows the fluorescent efficiency to be input along with other canopy parameters to determine the canopy fluorescent efficiency (see Equation 9 through Equation 13). The fluorescent efficiency of the leaf is first input into Equation 9. Since the formula incorporates changing efficiency with
canopy depth, leaf level fluorescence needs to be split in two. Canopy depth only partially influences the overall fluorescence from the canopy. Therefore the efficiency is broken into two parts. One part, “a” (see Equation 9), provides a base level fluorescence efficiency value for the canopy. The other part, “b”, is the amount of fluorescence efficiency changing with canopy depth. Olioso et al. (1992) gave “a” the value 0.94 and “b” the value 0.06. These parameters were multiplied by the quantum efficiencies at 690 nm and 730 nm to determine a new value for “a” and “b” (see Table 7). This allowed determination of the leaf level fluorescence at any canopy depth. The values of a and b are different for each ratio because the quantum efficiency values change when the ratio does.
Table 7: Fluorescent Efficiencies for Bands 690, 730 nm and Variables “a” and “b”

QE690 and QE730 represent the fluorescent efficiency (number of fluorescent photons per incident photon) for 1 nm in the 690 nm and 730 nm bandwidths

<table>
<thead>
<tr>
<th>Ratio</th>
<th>QE690 (*10^-5)</th>
<th>a (0.94) (*10^-5)</th>
<th>b (0.06) (*10^-5)</th>
<th>QE730 (*10^-5)</th>
<th>a (0.94) (*10^-5)</th>
<th>b (0.06) (*10^-5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.31 (Orig.)</td>
<td>3.13</td>
<td></td>
<td></td>
<td>9.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.35</td>
<td>13.34</td>
<td>(12.54)</td>
<td>(0.8)</td>
<td>9.88</td>
<td>(9.29)</td>
<td>(0.59)</td>
</tr>
<tr>
<td>1.15</td>
<td>11.36</td>
<td>(10.68)</td>
<td>(0.68)</td>
<td>9.88</td>
<td>(9.29)</td>
<td>(0.59)</td>
</tr>
<tr>
<td>0.8</td>
<td>7.91</td>
<td>(7.44)</td>
<td>(0.47)</td>
<td>9.88</td>
<td>(9.29)</td>
<td>(0.59)</td>
</tr>
<tr>
<td>0.6</td>
<td>5.93</td>
<td>(5.57)</td>
<td>(0.36)</td>
<td>9.88</td>
<td>(9.29)</td>
<td>(0.59)</td>
</tr>
<tr>
<td>1.18</td>
<td>11.66</td>
<td>(10.96)</td>
<td>(0.7)</td>
<td>9.88</td>
<td>(9.29)</td>
<td>(0.59)</td>
</tr>
<tr>
<td>0.97</td>
<td>9.59</td>
<td>(9.01)</td>
<td>(0.58)</td>
<td>9.88</td>
<td>(9.29)</td>
<td>(0.59)</td>
</tr>
</tbody>
</table>

The next concern was how much influence canopy depth has on changing the fluorescent efficiency. Since this parameter can vary due to canopy growth conditions it was decided to find the range of its influence. This was done by using more than one value for the variable “c” in Equation 9. This variable adjusts the increased amount of fluorescence due to lower canopy depths. To produce a range, two extreme values were decided upon. One of those values being zero. In this case the fluorescent efficiency does not change at all with canopy depth (see Figure 14). The other values for “c” being 0.5 for 690 nm and 0.7 for 730 nm (see Table 8). These other values provide an upper limit for the influence of canopy depth on fluorescence. The values of “c” are different for both 690 nm and 730 nm to take into account the different affects the canopy has at those two wavelengths.
Table 8: Input Parameters Used to Generate Four Canopy Fluorescent Efficiencies

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>small range ( \rho )</th>
<th>medium range ( \rho )</th>
</tr>
</thead>
<tbody>
<tr>
<td>690 nm</td>
<td>0.94</td>
<td>0.06</td>
<td>0.7</td>
<td>0.18</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>0.94</td>
<td>0.06</td>
<td>0.7</td>
<td>0.12</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>0.94</td>
<td>0.06</td>
<td>0.0</td>
<td>0.18</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>0.94</td>
<td>0.06</td>
<td>0.0</td>
<td>0.12</td>
<td>0.05</td>
</tr>
<tr>
<td>730 nm</td>
<td>0.94</td>
<td>0.06</td>
<td>0.5</td>
<td>0.18</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>0.94</td>
<td>0.06</td>
<td>0.5</td>
<td>0.12</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>0.94</td>
<td>0.06</td>
<td>0.0</td>
<td>0.18</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>0.94</td>
<td>0.06</td>
<td>0.0</td>
<td>0.12</td>
<td>0.05</td>
</tr>
</tbody>
</table>

The last parameter needed for modeling the canopy is the attenuation and interception coefficients. These values change depending on the canopy type in question (see Table 4). They also change depending on the chlorophyll concentration in the plant (see Table 5). The soybean plant was assumed to have a spherical canopy type. The reason for this is previous research has modeled the soybean canopy as a spherical canopy type (see Figure 16) [Olioso et al., 1992]. When modeling each plant condition the appropriate set of attenuation and interception coefficients were used from Table 5.

Soil reflectivity is another issue that can affect the signal received by a sensor. It was decided to use two different scenarios. The first being the soil reflection being known within a deviation of 20%. It was assumed that one could know the soil reflectivity of a crop within 20% of it’s actual value. This value could be reduced even more if a secondary measurement was taken to actually record the reflectivity of the soil.
This would enhance one’s ability to reduce the variability in the collection. The range that was picked for this case was a soil with a reflectivity of 12 to 18% (15% reflectivity with a 20% deviation from the expected value). The problem is the variation in soil reflectivity may not always be within such a small range.

For the cases where the soil may take on many different values a second scenario was used. In this case a larger range of soil reflectivity was implemented. The range that was utilized was from 5 to 20%. The basis for this range was made from Figure 28 [Gao et al., 1990].

![Figure 28: Typical Soil Reflectance Curves for Five Major Soil Types](image)

Typical soil reflectance curves for five major soil types [Condit, 1970; Stoner and Baumgartner, 1980]: (1) organic-dominated, moderately fine texture; (2) organic-dominated, moderately course texture; (3) iron dominated laterite-type soil; (4, 5) iron- and organic-rich soil, respectively. The vertical dashed lines represent the 0.94- and 1.14-μm water vapor band regions used in the retrievals. [Gao et al., 1990]

In the plot it can be seen that most soil types fit within this reflection range (when looking at wavelengths from 600 to 800 nm). Therefore it is assumed that this range represents an extreme range for the unknown reflectivity of soil. In the paper on the canopy model the range used was from zero to 50% [Olioso et al., 1992]. It was decided that this was excessive and for field conditions that range was actually of smaller
magnitude. It is assumed that this range covers such issues as the history of precipitation in the area at the time of collection. The amount of soil moisture will affect its reflectivity. Therefore soil type is not the only issue affecting its reflectivity. By using a larger range the variability due to precipitation history has more of a representation in the calculations.

The soil reflectivity values along with the other canopy variables used as input are listed in Table 8.

The range of parameters for “c” and “p” in Table 8 were used to create four different sets of values to model a canopy signal. This was done two separate times, once for a small and once for a medium range soil reflectivity. This generated four different canopy signals for a particular plant condition. The parameter sets were used to generate four canopy fluorescence efficiency curves at a particular plant condition. This was done at bands 690 nm and 730 nm for each plant condition under examination. Figure 29 shows an example of the curves generated for the 690 nm and 730 nm bands for a soybean plant with a chlorophyll concentration of 40 μg/cm².
Figure 29: Fluorescent Efficiency of the Canopy for Four Different Conditions at Bands 690 nm and 730 nm

The above plots are for a soybean plant with a leaf area ratio of 0.8 and a chlorophyll level of 40μg/cm². The sensor height was located at 300m. The soil reflectance used was for a medium range. The following values of “c” and “ρ” were used to generate the four separate curves (for 690 nm c=0, 0.5 and for 730 nm c=0, 0.7; in both cases ρ = 0.05, 0.2).

For 690 nm: red curve has c = 0.7 and ρ = 0.2, blue curve has c = 0.7 and ρ = 0.05, green curve has c = 0.0 and ρ = 0.2, and magenta curve has c = 0.0 and ρ = 0.05

For 730 nm: red curve has c = 0.5 and ρ = 0.2, blue curve has c = 0.5 and ρ = 0.05, green curve has c = 0.0 and ρ = 0.2, and magenta curve has c = 0.0 and ρ = 0.05

5.1.9 Prospect modeling of Plant Reflectivity

Since the sensor system records the amount of light coming from a plant canopy not all of it is fluorescent energy. Some of it is due to the reflection of light from off of the plant. The amount of reflectivity depends on the plant type and chlorophyll concentration of the plant. This reflection influences how much background noise is added to the fluorescent signal due to sun and sky light reflecting from the vegetation. Therefore reflectance values for the plant are needed. In order to determine these values the PROSPECT model was utilized. This model, developed by Jaquemoud and Baret, determines the reflectivity of a plant given certain input parameters [Jaquemoud et. al, 1990]. The input values needed are the chlorophyll concentration (C_{a+b}), water
concentration ($C_w$), and a structural parameter "N" (based on leaf thickness) of the plant. Another value needed is the number of degrees from normal one is viewing the plant (represented as "$\alpha$"). The input values used were characteristic of a soybean plant. The values used for the input are listed in Table 9.

<table>
<thead>
<tr>
<th>$C_w$</th>
<th>N</th>
<th>$C_{a+b}$</th>
<th>$\alpha$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0137</td>
<td>1.832</td>
<td>10 $\mu g$/cm$^2$</td>
<td>1°</td>
</tr>
<tr>
<td>0.0137</td>
<td>1.832</td>
<td>20 $\mu g$/cm$^2$</td>
<td>1°</td>
</tr>
<tr>
<td>0.0137</td>
<td>1.832</td>
<td>40 $\mu g$/cm$^2$</td>
<td>1°</td>
</tr>
<tr>
<td>0.0137</td>
<td>1.832</td>
<td>60 $\mu g$/cm$^2$</td>
<td>1°</td>
</tr>
</tbody>
</table>

The reflectance was desired for a nadir point of view which would have an "$\alpha$" of zero degrees. However the calculations of the reflectance model blow up for this input. This is why a value of one degree was used instead. It is assumed that this is close enough to provide a reasonable value for the plant reflectance at a nadir point of view.

Since the fluorescent signal for four different chlorophyll concentrations is desired the reflectance at each concentration needs to be determined. That is why the reflectance was found for four different levels of chlorophyll (10, 20, 40, and 60 $\mu g$/cm$^2$).

The above input parameters were used to find the reflectance of a soybean plant using the PROSPECT model. A computer program of this model, written by Paul Barnes [Barnes, 1995], was used to generate the reflectance curves at the four different chlorophyll concentrations. Figure 30 illustrates the change in soybean reflectance at the different concentrations.
The plot illustrates that as the chlorophyll level decreases the reflectance significantly increases. These curves were utilized in the radiometric calculation of background signal reaching the sensor.

5.1.10 Lowtran Modeling of the Atmosphere

Once the amount of fluorescence from a canopy is found the influence of the atmosphere on the signal needs to be determined. The atmosphere adds light from the sun and sky while also attenuating the signal from the plant through absorption and scattering of the signal. In calculating the signal reaching the sensor these affects have to be incorporated into the radiometric calculations. However, one has to first determine
the characteristics of a specific atmosphere. This was accomplished by utilizing the Lowtran model. This routine generates many different values for an atmosphere. The model is very robust in that it allows one to calculate the values of many different types of atmospheres.

In order to generate the output many decisions had to be made on the specific type of atmosphere to be modeled. Not all of the input parameters will be discussed here but they are located in the appendix. What follows is a discussion of the general atmosphere modeled. It was decided to model the atmosphere in the Georgia area (Latitude 34°, Longitude 86°) which is a reasonable area for the growing of soybean crops. It was decided to model a mid-summer day (day of year 182) at 5 PM in the afternoon with no rain present. The time of day was chosen due to the reduced amount of sun and skylight at that time of day yet there still being enough to keep the plant active photosynthetically.

The input variables were loaded into a program called Control 7. This program builds the card decks that are used in the Lowtran model. The specific values loaded into Control 7 are listed in the appendix (9.1). Control 7 was run three separate times. Once for each sensor height. The only input parameter to change was that of the sensor height (the value for the path distance is automatically determined when 0.0 is input for this variable). The program then produced a card deck for each sensor height. Control 7 is not a standard routine used with Lowtran. It was written at the Rochester Institute of Technologies Center for Imaging Science to be more user friendly in entering input data into Lowtran. The typical format of input into Lowtran are the card decks Control 7 produces. Therefore, the card decks produced by Control 7 are also listed in the appendix (see 9.2 through 9.4).

Lowtran produces a plethora of data on the atmosphere, however, not all of it was utilized. The following output values were the items of interest: sunlight at the exoatmosphere, skylight reaching the ground, atmospheric transmittance from the
exoatmosphere to the ground (needed to determine the amount of sunlight reaching the ground), the transmission of the atmosphere from the target to the sensor, and the upwelled radiance of the atmosphere in the target to sensor path. These output values were found for the three different sensor heights (10m, 30m, 300m).

The irradiance of the sun and sky light reaching the ground is illustrated in Figure 31. The irradiance of the sun reaching the ground was found by using Equation 24. The equation took the product of $E_{sun, \lambda}$ (from Figure 31) and $\tau_{atm, sun, \lambda}$ (from Figure 32) to obtain $E_{sun_{ground}, \lambda}$ (in Figure 31).

**Figure 31: Irradiance on the Ground from Solar and Sky Light**

![Figure 31: Irradiance on the Ground from Solar and Sky Light](image)

The transmission of the atmosphere from the exoatmosphere to the ground does not change for each sensor height and it is shown in Figure 32.
The transmission of the atmosphere from the target to the sensor is very similar for the distance of 10m and 30m. In fact it is very close to one. This makes sense as there is little atmosphere for the signal to travel through. At 300 m the loss of signal due to the atmosphere is more relevant as the transmission begins to become less than one. This trend is illustrated in Figure 33.
The amount of atmosphere between the sensor and target also influences how much upwelled radiance is present in the signal. Figure 34 shows that as the sensor height increases the amount of radiance from the atmosphere increases.
Figure 34: Upwelled Radiance for Three Different Sensor Heights (10, 30, and 300m)

5.1.11 Sensor Systems and Collection Scenarios

When looking to remotely collect laser induced fluorescence from vegetation the desired conditions can vary. One may want to try to assess the condition of their crops in a greenhouse while another may want to take a broad assessment of their entire farm. This creates the need to measure fluorescence at many different standoff distances. This often times requires an adjustment in the collection system for different distances. This research calculates the expected signals for the following three collection scenarios: measurement of plants in the greenhouse environment using a hand held collection device, measurement of localized plants in the field using a mobile truck based measuring device, and making widespread measurements of fields using an airborne (helicopter) based measuring device.
Due to the different collection tasks of all three devices, a different collection system is needed in each case. This research was conducted using the parameters from three such systems. The specifications for these systems were obtained from Special Technologies Laboratory in Santa Barbara California [Lutz, 1995]. Their work in collecting laser induced signals has led them to design three such sensor packages. This research used these parameters to aid in calculating realistic sensor signals for the three different systems.

The first system is a hand held sensor that can be used by a human operator at a standoff distance of 10 m. This system was designed to be used in areas such as greenhouses.

The second system was a truck based platform that was designed to make measurements at a distance of 30 m. The system was designed to make localized measurements of field crops.

The third system was a helicopter based platform that was designed to make measurements at a distance of 300 m. The system was designed to measure broad areas of vegetation.

There were several parameters that changed for each sensor height. These values were needed for the radiometric calculations used in this research. They are listed in Table 10.
Table 10: Radiometric Input Parameters for Three Sensor Platforms

<table>
<thead>
<tr>
<th></th>
<th>Hand Held</th>
<th>Truck Based</th>
<th>Helicopter Based</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser Energy (Q)</td>
<td>0.020 joules</td>
<td>0.070 joules</td>
<td>0.180 joules</td>
</tr>
<tr>
<td>Height (h)</td>
<td>10 meters</td>
<td>30 meters</td>
<td>300 meters</td>
</tr>
<tr>
<td>Projected Diameter(D)</td>
<td>1.95 meters</td>
<td>4.47 meters</td>
<td>13.44 meters</td>
</tr>
<tr>
<td>Focal Length</td>
<td>0.08 meters</td>
<td>0.105 meters</td>
<td>0.35 meters</td>
</tr>
<tr>
<td>Aperture Diameter</td>
<td>0.04 meters</td>
<td>0.053 meters</td>
<td>0.175 meters</td>
</tr>
<tr>
<td>Pulse Time</td>
<td>10 ns</td>
<td>10 ns</td>
<td>10 ns</td>
</tr>
<tr>
<td>Detector Size</td>
<td>0.0125 m (wide)</td>
<td>0.0125 m (wide)</td>
<td>0.0125 m (wide)</td>
</tr>
<tr>
<td></td>
<td>0.009525 m (long)</td>
<td>0.009525 m (long)</td>
<td>0.009525 m (long)</td>
</tr>
<tr>
<td>Resolution Element Size</td>
<td>3.77*10⁻⁹ meters²</td>
<td>3.77*10⁻⁹ meters²</td>
<td>3.77*10⁻⁹ meters²</td>
</tr>
</tbody>
</table>

Of course the first value, height, changes for each distance. The increased distance warrants the use of a higher power laser. This is to ensure the laser induced signal is larger enough to be seen at that remote location. The energy output by the laser is listed as the variable “Q”. It indicates the amount of joules output by the laser.

The focal length and aperture diameter are two other changes in each system. This change is necessary to properly collect the fluorescent signal at each distance. The standoff distance also influences the projected size of the detector on the ground. Therefore the projected size of the diagonal, from one corner of the detector to the other, will also be influenced by the standoff distance. The size of this projected diagonal is influenced by the collection optics, sensor standoff distance, and physical size of the detector. The objective was to make sure that the ground area measured by the detector was totally illuminated with laser light. Therefore the size of the projected diagonal was
necessary to determine the amount of divergence necessary in the laser beam. In order to
determine the divergence (see Equation 16) the projected diameter of the detector was
used as the spot size diameter of the laser light on the ground. This allowed the
excitation light to excite enough vegetation to produce a signal for the entire sensor.
Therefore the projected diameter of the sensor was taken as the needed diameter in the
laser spot size. This can be seen in Figure 20. The amount of spread in the laser beam is
affected by the excitation optics and the sensor standoff distance. The values for the
project diagonal at each distance are also listed in Table 10. The actual physical size of
the diagonal of the detector array was 0.0157 meters.

The detector used in the sensor system design was a Xybion camera, which is a
microchannel plate image intensified imaging detector [Lutz, 1995]. The detector array
had a rectangular shape and measured 0.009525 meters in length and 0.0125 meters in
width (the diagonal length was listed in the above paragraph). The size of one resolution
element from the array was $3.77 \times 10^{-9}$ meter$^2$. The camera sensitivity was also provided
by Special Technology Laboratories. Figure 35 shows the camera responsivity curve
over wavelengths in the region from 300 nm to 800 nm.
The curve shows the camera is more sensitive to the signal being collected at 690 nm versus that at 730 nm. This is going to have the effect of providing a higher response to input at the 690 nm band in comparison to the 730 nm band.

The collection system design utilizes an optics and filter system to collect light at the desired wavelengths. It was assumed in this research that the optics had a transmission of one. The transmission rates of the filters used to collect information at bandwidths 690 nm and 730 nm are illustrated in Figure 26. The effects of the filter transmission were taken into account in the radiometric calculations used in Equation 29.

The above specification were the main parameters utilized in determining the sensor system response to an incoming fluorescent signal.
5.1.12 Radiometric Calculations

The above sections describe different components used in the radiometric calculations described below.

When the fluorescence efficiency of the plant canopy (section 5.1.8) is known the next step is to determine how much excitation energy reaches the plant. Many of the factors listed above affect this value. The following discusses the specifics of the radiometric calculations listed in the theory section. It maps out the process used to determine the fluorescent signal at a sensor.

The first step of the process was to determine the divergence of the excitation laser of the system. Equation 16 was used along with the projected diameter on the ground ("D") and the sensor height ("h") for all three sensor platforms. The radiant intensity of the laser was then found utilizing Equation 18. The pulse time of the laser ("dT") was considered to be 10 ns. This appears to be a common laser pulse time used in past research of fluorescence.

In order to determine the irradiance ("E") reaching the plant the sensor height and transmission effects have to be known. The transmission of the atmosphere from the laser to the target was obtained from Lowtran (see Figure 33). At this point the only transmission value needed was at the laser’s excitation wavelength. This information was used in Equation 19.

The fluorescent efficiency of the canopy was then determined for the soybean plant. The canopy structure affects the canopy level fluorescence. Different canopy types affect leaf level fluorescent signals in different manors. Typically the more transparent a canopy the more it’s inner components add to the fluorescent signature. This adds to the overall variability in the fluorescent signal. Therefore to take into account the variability due to this factor a spherical canopy type was modeled. This canopy type tends to be more transparent to light. This canopy type was chosen for the following reason. If the calculations of a transparent canopy show the difference
between a healthy and stressed plant then the same should be true for a less transparent canopy. This follows because a less transmissive canopy will have less variability in the fluorescent signal. This should make it easier to discriminate between a healthy and stressed plant. The different attenuation and interception coefficients for the different canopy types are listed in Table 4.

There are many attributes that can affect a plant canopy’s fluorescence. Two such factors are soil reflectivity and the influence of the inner canopy. These factors add variability to the fluorescent signal. To take this variability into account four canopy efficiencies were determined. These efficiencies represent a range of possible values that a particular plant condition may demonstrate.

<table>
<thead>
<tr>
<th>Sensor Height</th>
<th>10 m</th>
<th>30 m</th>
<th>300 m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser Irradiance (E)</td>
<td>6.683 *10^5</td>
<td>4.436*10^5</td>
<td>1.213*10^5</td>
</tr>
</tbody>
</table>

This research was concerned with the fluorescent bands (690nm and 730 nm) reaching the sensor. When calculating the signal level (of 690 nm and 730 nm) for a particular ratio (690 nm/ 730 nm) the fluorescent efficiency curves took on four different shapes. The shapes of the curves (shown in Figure 29) are for the fluorescent efficiency of the canopy over varying leaf area index. The four separate curves (for each band) represent a range of possible efficiencies (mentioned above) due to varying soil reflectivity and rate of increasing fluorescence with canopy depth. The input variables that adjust these curves are “ρ“ and “c” and they are listed in Table 8.

The varying fluorescent efficiencies were then combined with the laser irradiance (Table 11) for varying sensor heights. This provided the range of possible canopy
fluorescent signals. It was assumed there was no solar induced fluorescence present in the canopy fluorescence. The result of this step provides the radiant excitation of fluorescence from the plant for varying leaf area index. Assuming the plant is a lambertian emitter [see section 2.5.4] the radiant excitation can be converted into a fluorescent radian value for the plant by dividing the radiant excitation by \( \pi \) (see Equation 23). See an example of the resulting radian plots in Figure 36.

**Figure 36: Fluorescent Radiance Leaving Soybean Plant for 4 Canopy Parameters**

![Radiance plots](image)

The above plots are for a soybean plant with a leaf level ratio of 0.8 and a chlorophyll level of 40\( \mu \)g/cm\(^2\). The sensor height was located at 300m. The soil reflectance used was for a medium range. The following values of “\( c \)” and “\( p \)” were used to generate the four separate curves (for 690 nm \( c=0 \), 0.5 and for 730 nm \( c=0 \), 0.7; in both cases \( p = 0.05, 0.2 \)).

Knowing the radian due to fluorescence into the hemisphere above the plant, the next step was to determine the signal reaching the sensor. In order to find this the amount of background signal had to be calculated (see Equation 26). Using Lowtran the upwelled and downwelled radiance values were determined for all three sensor heights (see 5.1.10). The amount of downwelled radiance reflected back towards the sensor depends on the plant reflectivity. The reflectivity of the plant, at a certain chlorophyll concentration, (see Figure 30) was used to determine the amount of downwelled radiance
reflected towards the sensor. The amount of this reflected sun and sky light reaching the sensor was found by taking into account the transmission of the atmosphere. Depending on the height of the sensor, one of the curves from Figure 33 was used to determine the amount of signal loss due to the atmosphere. This provided the downwelled portion of background radiance. The upwelled radiance was then added to the background signal. The height of the sensor dictated which one of the curves from Figure 34 was used as the upwelled radiance. The upwelled and reflected downwelled radiance reaching the sensor provided the background signal.

In order to determine the signal reaching the sensor, the fluorescent signal and background signal had to be added together. First the amount of fluorescent radiance, from the plant canopy, reaching the sensor had to be found. Therefore the amount of signal loss due to the atmosphere was needed. Again depending on the sensor height one of the atmospheric transmission curves from Figure 33 was utilized. The fluorescent, reflected, and upwelled radiance values (at the sensor) were then added together (see Equation 27).

Knowing the amount of radiance reaching the sensor platform, the amount actually reaching the detector had to be found. The sensor takes up a certain fraction of the hemisphere above the plant. The amount of radiance entering the sensor depends on the collection optics of the system. Equation 28 and Equation 29 from the radiometric theory section are used for this characterization. The desired value, G#, is found from the focal length, aperture diameter, and sensor transmission of the system. The focal length and aperture diameter are different for each platform height (see Table 10). They regulate the amount of light entering the system. The sensor transmission affects how much of the entering radiance actually makes it to the detector.

The sensor transmission is made of the optics transmission (which was assumed to be one) and the transmission of the filters. The curves in Figure 26 show the filter transmission rates. The product of the optic and filter transmission are taken to find the...
sensor transmission. In this case, due to the assumption, the output is just the filter curves.

Having obtained the radiance reaching the sensor ("Lsensor") and the sensor characteristics ("G#"), the irradiance on the detector was found (by using Equation 30). The division of the radiance by a term that includes collection area causes the result to be the irradiance reaching the sensor.

At this point it was desired to find the radiant flux. As seen in Equation 31 this depends on the size of one detector resolution element. The size of the detector element is listed in Table 10. This provides the radiant flux on the detector. However the quantum efficiency response of the camera was known per incident photon. Therefore radiant flux needed to be converted to the number of photons reaching the sensor. The input flux reaching the detector varies over wavelength. Therefore the conversion to photons was implemented over the wavelengths reaching the sensor. The spectral information was also needed due to the camera not having a linear response to all wavelengths (see Figure 35). Another factor in the camera signal is the camera exposure time. The length of time the system is shuttered to collect light influences the number of photons reaching the sensor. In Equation 32 the gate of the system is utilized. The time of the gate also influences the amount of background photons reaching the sensor. Thus the longer the gate the less fluorescent photons are measured compared to the background signal. This is due to the fact that active fluorescence decreases over time while the background radiance is consistent (when dealing with short time intervals). Therefore a short gate was chosen. It was decided that 10 ns was a reasonable collection time for the fluorescent signal. An assumption was made with the gate time. That assumption was that all of the fluorescent energy emitted from the plant, due to the laser pulse, is collected by the sensor. Figure 37 shows the number of photons reaching a detector resolution element for four different canopy curves (at one plant condition).
Figure 37: Total # of Photons Reaching the Detector for 4 Canopy Parameters

The above plots are for a soybean plant with a leaf level ratio of 0.8 and a chlorophyll level of 40μg/cm^2. The sensor height was located at 300m. The soil reflectance used was for a medium range. The following values of “c” and “ρ” were used to generate the four separate curves (for 690 nm c=0, 0.5 and for 730 nm c=0, 0.7; in both cases ρ = 0.05, 0.2).

With the number of photons reaching the detector calculated the signal from the detector was found. This was done by integrating the product of detector quantum efficiency and photon count over the bandwidth of interest. The camera quantum efficiency used is shown in Figure 35. The integration was actually conducted as a summation with a 1 nm interval over the wavebands at 690 nm and 730 nm. The camera signal was determined for both the 690 nm and 730 nm bands.

The resulting signal at bands 690 nm and 730 nm is illustrated in Figure 38 for one theoretical situation. When comparing the number of incident photons in Figure 37 to the signal in Figure 38 one can see the effect the detector responsivity has had on the result. It has preferentially boosted the signal at 690 nm.
The above plots are for a soybean plant with a leaf level ratio of 0.8 and a chlorophyll level of 40μg/cm². The sensor height was located at 300m. The soil reflectance used was for a medium range. The following values of “c” and “p” were used to generate the four separate curves (for 690 nm c=0, 0.5 and for 730 nm c=0, 0.7; in both cases p = 0.05, 0.2).

The strength of the signal relative to the noise was desired to determine if the signal could even be detected much less discriminated from subsequent measurements. To do this the signal to noise ratio (SNR) of the detector was found using Equation 34. The SNR was of the camera was found using an Fcam value of 1.4 and a read noise of 12 photoelectrons (from one resolution element). These were characteristic values for the Xybion camera. The read noise of the camera is the output of the camera when there is zero input into the camera. The Fcam component is the noise added by the camera due to photons incident on the detector and the amplification of the signal. This value characterizes the noise response of the camera and was measured by Special Technology Laboratories in California. These values were implemented along with the incoming signal to determine SNR. Figure 39 illustrates the SNR over a range of leaf area index for one plant canopy.
The amount of noise added to the signal from the camera was then found from the SNR. This was accomplished by dividing the signal by the SNR (see Equation 35). This provided the noise (standard deviation) of the camera output from the signal. This provides information on how much variation can be expected due to the detector. If there is too much variation due to noise the differences between plant conditions may not be visible. If this is the case pixel averaging can be utilized to help reduce the amount of variation (noise) in the signal. The more pixels averaged the lower the noise. The term “n” in Equation 35 accounts for the number of pixels averaged and reduces the noise accordingly.

The whole object of this work was to find the ratio of fluorescence at 690 nm and 730 nm. To find this the signal at 690 nm was divided by the signal at 730 nm. This provided an index as to the plants condition. The index was found over the varying leaf area index values for the plant. This is illustrated in Equation 36.

The above plots are for a soybean plant with a leaf level ratio of 0.8 and a chlorophyll level of 40μg/cm². The sensor height was located at 300m. The soil reflectance used was for a medium range. The following values of “c” and “ρ” were used to generate the four separate curves (for 690 nm c=0, 0.5 and for 730 nm c=0, 0.7; in both cases ρ = 0.05, 0.2).
It was necessary to find the standard deviation in the index value next. The signals at 690 nm and 730 nm were both found to have a variance due to camera noise. This variance in the bands affects the variance of the ratio. The standard deviation of the index value was determined with Equation 38. The basis of this equation is discussed in the theory section.

The range of expected index values was then found as illustrated in Figure 40.
Figure 40: Index and Range Associated with All Four Canopy Parameter Sets

The above plot is for a soybean plant with a leaf level ratio of 0.8 and a chlorophyll level of 40μg/cm². The sensor height was located at 300m. The soil reflectance used was for a medium range. The following values of “c” and “p” were used to generate the four separate curves (for 690 nm c=0, 0.5 and for 730 nm c=0, 0.7; in both cases p = 0.05, 0.2). The standard deviation and ranges (due to the standard deviation) for all four cases is also shown. The variation is after pixel averaging over 16 resolution elements.

This plot shows the indexes and standard deviation of four input signals. These four inputs take into account the expected range of values from one plant sample. As mentioned earlier each of the four curves represents the canopy signal changing due to
variances in soil reflectivity and changes in the influence of canopy depth on the signal. These four curves illustrate changes in the index (of one plant condition) due to canopy factors influencing the fluorescent efficiency of the plant. These changes in the canopy factors cause a variation in the expected signal. These values are accounted for because in real fluorescent collections the specific canopy conditions will not be known. This shows not only that the camera noise add variation to the index so does the variance in the fluorescent canopy signal. The first step in using this information was to average the index value for these four input curves. This provides the mean expected index value for varying canopy characteristics. The equation for this was Equation 39 in the radiometric theory section.

The mean index provides a reasonable expected signal however the standard deviation due to the four input signals causes the variation in the index to rise. To take this into account the standard deviation in the index due to the input signals was determined. Equation 40 was used to determine this value. This does not include the deviation due to the camera noise.

At this point the standard deviation, due to input variation, of the mean index was known. The variance in the index due to camera noise had to be incorporated next. Each one of the four canopy signals produced a certain camera noise. The four canopy signals were averaged to provide an expected mean signal from a plant canopy. Therefore it was desired to obtain a typical output of camera noise. To do this a simple average of the camera signal variation in all four curves was taken. Equation 41 shows the applied formula.

There were basically two forms of index variation dealt with here. One being the variation due to signal input. The other due to camera response. In order to properly compare index values an overall variation in the signal had to be found. This was done by combining the noise from the two sources of variation in the index value. Since noise values add in quadrature the method in Equation 42 was used.
Figure 41: Average Index (and Standard Deviation) from All 4 Canopy Parameter Set Signals

The above plot is for a soybean plant with a leaf level ratio of 0.8 and a chlorophyll level of 40µg/cm². The sensor height was located at 300m. The plot is the average output from four different canopy parameters. The four parameters sets were as follows: The soil reflectance used was for a medium range. The following values of “c” and “p” were used to generate the four separate curves (for 690 nm c=0, 0.5 and for 730 nm c=0, 0.7; in both cases p = 0.05, 0.2). The variation is after averaging over 16 resolution elements.

This provided an overall index value for a particular plant condition and its relative variation. An example of this is illustrated in Figure 41. All that was left was to find if the index of one plant condition was separable from another. To do this the above steps were repeated for a plant with a different leaf level ratio (690 nm/730 nm). This provided data on soybean plant under two different health states. The last issue became how to decide if the separation between the two was large enough to be distinguished from one another.

In order to accomplish this Thurstones complete law of comparative judgment was used [Gescheider, 1985]. Found in Equation 45, the equation uses a z-score to aid in finding the minimum separation needed between two curves in order to separate them from one another. The level of the z-score depends on what percent reliability does one
want to distinguish the two curves. In this research a confidence level of 85% was used (z-score 1.04).

The decision on whether one canopy condition can be seen from another will be based on certain canopy densities. The LAI index from 2 to 6 will be the main canopy densities of interest. This is due to the fact that the LAI of soybean is typically within this range during the growing season (see Figure 27). The results for younger plants will be presented but conclusions will be based on the fact the soybean is at least a month old (has a LAI of 2 or more).
6. Results
The following is a discussion on the results from the input values and calculations mentioned earlier. It will involve an analysis of the signal to noise ratio and ability to distinguish different plant conditions. First the change in the leaf level index ratio will be discussed.

The theoretical soybean plant used in this research was analyzed at six different plant conditions. Each one of these plant conditions had a specific index ratio at the leaf level that was determined from previous research in the field. The six different leaf level ratios are listed in Table 6. As discussed in the approach (see section 5.1.6) this ratio was used to identify what quantum efficiency values were needed to model the ratio. In this research the ratio at the leaf level is not affected by the leaf area index of the plant. In reality a plant with a growing leaf area index is usually accompanied with an increasing chlorophyll concentration in the plant. This would cause the ratio at the leaf level to change with a growing plant canopy. However, in this research the chlorophyll concentration and ratio were assumed to be constant. In Figure 42 the variable "ratioQE" represents the ratio at the leaf level of the plant. It shows a straight line across increasing leaf area index for the reasons sighted above.

When the index for the entire canopy is measured the leaf area index begins to alter the expected signal ratio. Figure 42 shows that as the canopy begins to become more dense the index drops. The reason for this is two fold. In the first case, the fluorescence from deeper canopy leaves boosts the 690 nm signal more than the 730 nm signal (see section 3.9). When the canopy becomes more dense the measured fluorescent signal is made up of less information from deeper canopy levels. This is due to the canopy attenuating more light at higher canopy levels due to the more dense foliage. Therefore the 690 nm band is no longer getting the extra signal (relative to 730 nm) so the ratio begins to drop.
The second case is that the leaf absorption at 730 nm is less than for the waveband 690 nm [Olioso et al., 1992]. That is why the attenuation coefficients are smaller at 730 nm (see Table 4 and Table 5). So as the plant develops a larger canopy not only does the influence of inner canopy depths become smaller but the 690 nm band is affected more than the 730 nm band. This is due to the fluorescent energy in the canopy at 690 nm being attenuated more than the fluorescence leaving at 730 nm.

This drop in the index is illustrated in Figure 42 for four different canopy situations. The curves are represented by variables “ratio_{plant}”, “ratio_{a,plant}”, “ratio_{b,plant}”, and “ratio_{c,plant}” These curves represent the range of possible signals from a canopy due to varying soil reflectivity and rate of fluorescence at inner canopy depths. In each case it can be seen that the index of the plant drops quickly when increasing from low leaf area index values. The variation in the four curves also begins to spread out as the variability due to the four parameter sets becomes apparent. However, after a leaf area index of two is reached the change in the index begins to level off and the four parameter sets begin to level off in their variability of the signal.

The drop in the index from “ratioQE” is due to the affects of the canopy on the leaf level fluorescent signal. As stated above it is due to the light energy at 690 nm being attenuated more by the canopy at 690 nm than at 730 nm. Therefore when the index is viewed at the canopy versus the leaf level it drops in value (especially at higher leaf area indexes).

The next set of index values represent the number of photons, in each band, reaching the sensor system. They are the variables “ratio_{photons}”, “ratio_{a,photons}”, “ratio_{b,photons}”, and “ratio_{c,photons}” The further drop in the index value is influenced by many factors at this point. Atmospheric transmission, solar radiance, sky radiance, and plant reflectivity all influence the number of photons reaching the sensor at each band.

The amount of solar radiance appears to be slightly higher in the 690 nm band than over the 730 nm band (see Figure 31). There appears to be more upwelled radiance
in the 690 nm band than at the 730 nm band for a sensor height of 300m. In the 10 m and 30 m cases it appears more equivalent (see Figure 34). Even the atmosphere is more transmissive to the energy in the 690 nm band that at the 730 nm (especially at a sensor height of 300 meters). This would all seem to point to the fact the index should increase rather than decrease. Therefore the reflectivity of the plant must be the factor causing this decrease.

In fact when looking at Figure 30 it is obvious there is clearly a much higher reflectance at the 730 nm band than at the 690 nm band. Therefore any of the background energy adding to the detected signal (sun and sky light) will be reflected off the plant with more intensity at the 730 nm band. This is what causes the overall plant index to drop when at the sensor platform. The last factors affecting the ratio are from the sensor system itself.

The two parts of the system affecting the ratio are the collection optics and the sensor responsivity. The part of the collection optics dealt with in this research were the collection filters. The bandpass and transmission rates of each of these filters are comparable (see Figure 26). Therefore the filter system had little affect on the overall index ratio. The detector of the system did not have a flat response across the wavelengths in question. When viewing Figure 35 it is apparent that the camera is more sensitive to a signal from the 690 nm band than from the 730 nm band. This causes the resulting measured index to be increased way above the initial leaf level signal. This is illustrated in Figure 42 by variables “ratio_{electrons}”, “ratioa_{electrons}”, “ratiob_{electrons}”, and “ratio_{electrons}”

The resulting measurements from this system produce a much higher index value at a remote distance than at the leaf level. If this is not realized the performance of the system can be mistaken as a high ratio due to a change in plant condition. A plant may then be miss diagnosed as undergoing a stress.
The above plot is for a soybean plant with a leaf level ratio of 0.8 and a chlorophyll level of 40 μg/cm². The sensor height was located at 300 m. The soil reflectance used was for a medium range. The following values of “c” and “p” were used to generate the four separate curves (for 690 nm: c=0, 0.5 and for 730 nm: c=0, 0.7; in both cases p = 0.05, 0.2). RatioQE is the original ratio of quantum efficiencies at the leaf level. Ratio_plant is the ratio at the canopy level. Ratio_photons is the ratio of electrons at 690 nm and 730 nm reaching the detector surface. Ratio_electrons is ratio of the detector signal at 690 nm and 730 nm.
Knowing the overall affect on the index at the sensor system does not answer all the question raised in this research. First off the question becomes can the signal even be detected at the remote location in question. This was answered by determining the signal to noise ratio for the sensor system and plant condition in question.

The signal to noise ratio was determined at all three sensor platforms for all six plant conditions (this was repeated for two different soil reflectivity sets). This produced 36 SNR plots; six are illustrated here. The plots illustrate the signal to noise ratio for four separate canopy parameters (for bands 690 nm and 730 nm) for a healthy and DCMU stressed soybean plant. Figure 43, Figure 44, and Figure 45 show this for the sensor platforms at heights 10, 30, and 300 meters.

In each case it can be seen that the affect of the stress was to cause the four curves at 690 nm to be boosted in SNR. However, the band at 730 nm remains the same. This makes sense due to the way the quantum efficiencies were determined. The leaf level efficiencies at 730 nm were assumed to remain the same, therefore there is not a change in the SNR (for 730 nm) between the two plant conditions. When under DCMU stress the index at the leaf level increases. With a steady 730 nm band this meant the fluorescent efficiency at 690 nm had to be increased to accommodate this increase in the ratio. This produced a higher signal to noise ratio for the 690 nm band under a DCMU stress. This makes sense because a stress typically induces a higher fluorescent efficiency which in turn provides a higher signal level to be measured by the sensor.

What can also be seen through Figure 43, Figure 44, and Figure 45 is that the SNR decreases with increasing standoff distance. One reason for this is the systems designed for further collection distance are not able to excite the plant with as much laser light. Therefore the systems in closer proximity to the plant are able to produce a higher fluorescent output by the plant. Also the further away the system is from the plant the more the signal is attenuated due to the atmosphere (see Figure 33). The more
atmosphere between the plant and the sensor, the lower the signal level reaching the
detector.

Another issue to note is the SNR increase with increasing leaf area index. This is
especially true at lower LAI. This is due to the fact the plant material is providing the
signal. The higher the LAI, the more plant material and the stronger the fluorescent
signal from the plant. This increase tends to level off after the canopy has reached a leaf
area index value of 2.

The figures illustrate that the signal to noise ratio is high enough at all three
sensor heights to say the fluorescent signal can be detected. The lower level of SNR at
300 meters brings to question how much higher a system could go and still detect the
fluorescence. The SNR for the 730 nm at a 300 meter standoff drops just below 3. Any
lower of an SNR and detection of the 730 nm band would prove difficult. The SNR at
300 meters is significantly lower than for the other two systems which are at much closer
ranges to the plant. If longer ranges (significantly larger than 300 meters) of system
standoff were desired a higher intensity laser would have to be utilized.
Figure 43: Signal to Noise Ratio for a Healthy and DCMU Treated Soybean Plant at a 10 meter Standoff
Figure 44: Signal to Noise Ratio for a Healthy and DCMU Treated Soybean Plant at a 30 meter Standoff
Figure 45: Signal to Noise Ratio for a Healthy and DCMU Treated Soybean Plant at a 300 meter Standoff

Knowing that the signal can be detected still does not answer the question: Can the differences between two plant conditions be detected at a remote location? The fluorescent signal may be able to be measured but it was found the signal levels were too low and much signal variation added by camera noise. The noise level was too great to allow the differences between two plant conditions to be distinguished. This can be seen in Figure 46. This is the same plot as in Figure 40. The only difference between the two figures is the number of pixels averaged to lower the noise in the signal. In Figure 46 the index and variations is from one resolution element. In Figure 40 the index and variation are from the average of 16 resolution elements.
Figure 46: Index and Range of Soybean at 300 meters Without Pixel Averaging

The above plot is for a soybean plant with a leaf level ratio of 0.8 and a chlorophyll level of 40μg/cm². The sensor height was located at 300m. The soil reflectance used was for a medium range. The following values of "c" and "p" were used to generate the four separate curves (for 690 nm c=0, 0.5 and for 730 nm c=0, 0.7; in both cases p = 0.05, 0.2). The standard deviation and ranges (due to the standard deviation) for all four cases is also shown. The variation without any pixel averaging, noise is due to one resolution element.
When the results were first viewed it was evident that the noise added to much variation to allow discrimination of different plant conditions. This was the case for all three sensor platforms (10m, 30m, and 300m). It was decided that pixel averaging would then be necessary to be able to distinguish between different fluorescent ratios.

The next step was too decide how much averaging was necessary. The signal levels (and thus SNR values) were different at each standoff distance (see Figure 43 through Figure 45). Therefore each sensor platform would require different amounts of pixel averaging to account for the camera noise.

The question used in determining how many pixels to average together was the following: Can the different plant conditions be distinguished with a 85% confidence level? The process started by averaging over a small number of pixels and then increasing the number until the above goal was met. Typically pixel averaging is conducted over a square area (kernel). Therefore the number of pixels averaged was incremented in values that only form square kernels over the detector. The number of resolution elements averaged was increased from 4 to 9 to 16 until the above conditions were met. Therefore the results presented in this paper have undergone some amount of pixel averaging. The amount of resolution elements averaged at each sensor height is listed in Table 12.

| Table 12: Number of Resolution Elements Averaged at Each Sensor Platform |
|-----------------------------------------------|-----------------|-----------------|-----------------|
| Sensor Height                | 10 meters      | 30 meters      | 300 meters     |
| Resolution Elements Averaged = n | 4              | 4              | 16              |

So now back to the question; can the differences between two plant conditions be detected at a remote location? In order to answer this the indexes of different plant conditions were compared to each other (for a particular sensor height). Figure 47 shows
one of these comparisons. The figure shows the index of a soybean plant before and after a DCMU herbicide stress. The comparison was done for a system with a 10 meter standoff. The soil background was considered to have a small range in reflectivity. The plot also contains the range associated with each index value. The range is determined from the standard deviation in the index value. The range was found by subtracting and adding the standard deviation with relation to the index. The figure illustrates that added stress causes the index to increase.

It can also be seen that the two index values are separated from one another (averaging over four resolution elements). The ranges only overlap for leaf area index values below two. Above a LAI of two the ranges are close to one another but never overlap.

As mentioned earlier soybean plants typically have a LAI from 2 to 5.5 (see 5.1.7). Therefore this range in LAI was used when deciding if two plant conditions could be discriminated. When looking at index values in this range the two conditions were discriminated from one another with a statistical confidence level of 85%. Figure 48 shows this fact. The plot illustrates two curves. One curve is the statistical minimum difference needed to separate two distributions. The distributions being representative of the index and standard deviation of the two plant conditions. Equation 42 was used to determine this value over varying leaf area index. The second curve was the actual separation between the index values for the healthy and herbicide stressed plant. Figure 48 shows that the actual difference between the plant conditions is larger than the minimum needed to be able to separate the two index values. Only for LAI values below one were the conditions indistinguishable. So for an LAI above two with an 85% confidence level the conditions were separable. This was the decision process used to analyze the rest of the data.

Figure 49 and Figure 50 illustrate the same plant situation as above except the soil was considered to have a medium range in soil reflectivity. This case was found to
produce very similar results as for a small soil reflectivity range. In fact the differences in the overall index values are small enough to make the figures here look almost identical. So in the medium reflectivity case the two conditions are able to be separated (with an LAI above 2).

**Figure 47: Index and Range for a Healthy and DCMU Treated Soybean Determined at 10 meters with a Background Soil with a Small Reflectivity Range**

Index, range, and standard deviation for a healthy and DCMU stressed soybean plant with a chlorophyll concentration of 40 µg/cm^2. The blue curves represent the information for a healthy soybean plant. The red curves represent the information for the DCMU treated soybean plant.
Figure 48: Minimum and Mean Differences Between Indexes from a Healthy and DCMU Treated Soybean Plant Using a 10 meter Standoff (Small Soil Reflectivity Range)

The red curve represents the minimum separation necessary to distinguish the two conditions. The blue curve is the actual separation in index value between the two conditions.
Figure 49: Index and Range for a Healthy and DCMU Treated Soybean Determined at 10 meters with a Background Soil with a Medium Reflectivity Range

Index, range, and standard deviation for a healthy and DCMU stressed soybean plant with a chlorophyll concentration of 40 μg/cm². The blue curves represent the information for a healthy soybean plant. The red curves represent the information for the DCMU treated soybean plant.
The red curve represents the minimum separation necessary to distinguish the two conditions. The blue curve is the actual separation in index value between the two conditions.

The question that comes to mind is why there wasn’t a more significant difference between the results for the two soil reflectivity ranges. When looking at the range in canopy level fluorescent signals, the soil reflectivity is the parameter that causes the most variability in the signal reaching the sensor. The variability of the input signal does increase between a small and medium range soil reflectivity. However this increase is masked by the larger variability in the index value due to camera noise.

As can be seen in Figure 51 the variability in the plant signal (green curve) adds little to the overall variability (red curve) in the index value. The variability due to camera noise is the main contributor (blue curve).
Figure 51: Standard Deviation from the Index of a DCMU Treated Soybean Plant

![Graph showing Standard Deviation from the Index of a DCMU Treated Soybean Plant](image)

Standard Deviation values for a Soybean plant treated with a DCMU herbicide stress and measured at a distance of 10 meters. The red curve represents the standard deviation in the index due to camera noise. The green curve represents the standard deviation due to the input canopy signal. The blue curve is a combination of the two curves.

At 10 meters, the differences between the different chlorophyll levels (10, 20, 40, and 60 μg/cm²) were distinguishable by looking at the index values. Figure 52 and Figure 53 illustrate that the index values from each chlorophyll level are clearly separable from one another. The only time the values come close is when comparing the difference between 10 μg/cm² and 20 μg/cm². At low leaf area index values the range associated between the two indexes overlaps and below a LAI of one the conditions are indistinguishable. Figure 53 shows that the other conditions are separable at almost all canopy densities. In all cases the different plant conditions can be seen (with an LAI above 2).
Figure 52: Index and Ranges for Different Soybean Chlorophyll Levels and Two Different Soil Reflectivity Ranges Determined for a 10 meter Standoff

The red curves are the index and range for a soybean plant with a chlorophyll concentration of 10 μg/cm². The blue curves are the index and range for a soybean plant with a chlorophyll concentration of 20 μg/cm². The green curves are the index and range for a soybean plant with a chlorophyll concentration of 40 μg/cm². The magenta curves are the index and range for a soybean plant with a chlorophyll concentration of 60 μg/cm².
Figure 53: Minimum and Actual Differences between Indexes for Different Chlorophyll Levels of Soybean Determined for a 10 meter Standoff

Plots for a small range in soil reflectivity are listed on the left. Plots for a medium ranges in soil reflectivity are listed on the right. The red curve represents the minimum separation necessary to distinguish the two conditions. The blue curve is the actual separation in index value between the two conditions.

When measuring the index value at a 30 meter standoff the range of values associated with an index value become greater (also averaged over four resolution elements). This is in part due to the lower signal level reaching the camera. Therefore
the increased noise compared to signal adds to the variability in the index. This
increased range in the index values can be seen in the following plots of index values
determined for a 30 meter standoff system.

Figure 54 and Figure 56 show that the index (at 30 meters) does indeed have a
larger range than when compared with Figure 47 and Figure 49 (at 10 meters). In fact
the ranges in index overlap at all LAI values. Figure 55 and Figure 57 show that the
wider range still does not adversely affect the ability to distinguish between these two
plant conditions. Only for leaf area indexes below 2 does there appear to be too little of
an actual difference between index values to see a difference. In this case a LAI below 2
is small enough where it can be assumed fluorescent measurements would not typically
occur at such a stage of plant growth.
Figure 54: Index and Range for a Healthy and DCMU Treated Soybean Determined at 30 meters with a Background Soil with a Small Reflectivity Range

Index, range, and standard deviation for a healthy and DCMU stressed soybean plant with a chlorophyll concentration of 40 μg/cm². The blue curves represent the information for a healthy soybean plant. The red curves represent the information for the DCMU treated soybean plant.
Figure 55: Minimum and Mean Differences Between Indexes from a Healthy and DCMU Treated Soybean Plant Using a 30 meter Standoff (Small Soil Reflectivity Range)

The red curve represents the minimum separation necessary to distinguish the two conditions. The blue curve is the actual separation in index value between the two conditions.
Figure 56: Index and Range for a Healthy and DCMU Treated Soybean Determined at 30 meters with a Background Soil with a Medium Reflectivity Range

Index, range, and standard deviation for a healthy and DCMU stressed soybean plant with a chlorophyll concentration of 40 μg/cm². The blue curves represent the information for a healthy soybean plant. The red curves represent the information for the DCMU treated soybean plant.
Figure 57: Minimum and Mean Differences Between Indexes from a Healthy and DCMU Treated Soybean Plant Using a 30 meter Standoff (Medium Soil Reflectivity Range)

![Graph showing minimum and mean differences]

The red curve represents the minimum separation necessary to distinguish the two conditions. The blue curve is the actual separation in index value between the two conditions.

Figure 58 shows that the overlap of ranges at lower LAI levels is also present for indexes at different chlorophyll concentrations. Again the overlap is not severe enough or at large enough leaf area indexes to effectively be a problem. The curves in Figure 59 show that the only time two conditions cannot be distinguished is at very low LAI. In the case between 10 to 20 μg/cm² the conditions are indistinguishable for slightly larger LAI values (a value of ~1). The fact that these two conditions are harder to separate at this point shows itself in Figure 58. The plots indicate the ranges overlap to a greater extent than when measured from a 10 meter standoff.

This information provides the support that the system design for a 30 meter standoff could effectively distinguish between these six different plant conditions (with a LAI above 2).
Figure 58: Index and Ranges for Different Soybean Chlorophyll Levels and Two Different Soil Reflectivity Ranges Determined for a 30 meter Standoff

The red curves are the index and range for a soybean plant with a chlorophyll concentration of 10 μg/cm². The blue curves are the index and range for a soybean plant with a chlorophyll concentration of 20 μg/cm². The green curves are the index and range for a soybean plant with a chlorophyll concentration of 40 μg/cm². The magenta curves are the index and range for a soybean plant with a chlorophyll concentration of 60 μg/cm².
Figure 59: Minimum and Actual Differences between Indexes for Different Chlorophyll Levels of Soybean Determined for a 30 meter Standoff

Plots for a small range in soil reflectivity are listed on the left. Plots for a medium ranges in soil reflectivity are listed on the right. The red curve represents the minimum separation necessary to distinguish the two conditions. The blue curve is the actual separation in index value between the two conditions.

In the third case the system platform was at a 300 meter standoff. As discussed before longer standoff ranges produce lower fluorescent signals. This causes the range for an index to increase due to increased camera noise in relation to signal content. This
is illustrated in the following figures showing index values for different plant conditions. In this case to reduce some of the variation due to camera noise the signal was averaged over 16 resolution elements.

Figure 60 and Figure 62 illustrate the separation between the index before and after a DCMU herbicide stress. In the figures the range of index values, for both conditions, overlap over all leaf area index values. This indicates that it is becoming increasingly difficult to separate the two index values. Figure 61 and Figure 63 show that detecting a difference between a healthy and DCMU soybean plant is difficult to do at this range.

The figures show that only soybean plants with a leaf area index slightly above two can be discriminated from one another. At these higher LAI values the actual difference between index values is just distinguishable. In fact in the case of a larger range in soil reflectivity an LAI of slightly larger than 2.3 is needed to discriminate plant conditions (see Figure 63).

The lower signal level, due to the longer standoff distance, causes all the sources of variability to have more of an impact on distinguishing between indexes. For this reason the variability in the canopy signal plays a larger part in the distinguishing between two plant indexes. Therefore the difference between a small and medium range in soil reflectivity becomes more apparent. It still does not play a large roll, however, it's impact is evident in Figure 61 and Figure 63. When comparing the two plots, the medium range in soil reflectivity calls for a slightly larger difference in the index values in order for the two plant conditions to be distinguishable. While the medium range needs to have a LAI above 2.3, the small range can distinguish indexes for LAI values two and above. It is a subtle change, however, in a situation were the detectability of the stress is pushing the limits smaller factors take more precedent.

As long as one is measuring a soybean canopy with a leaf area index larger than 2.3 the herbicide stress is detectable at this standoff distance.
Figure 60: Index and Range for a Healthy and DCMU Treated Soybean Determined at 300 meters with a Background Soil with a Small Reflectivity Range

Index, range, and standard deviation for a healthy and DCMU stressed soybean plant with a chlorophyll concentration of 40 μg/cm². The blue curves represent the information for a healthy soybean plant. The red curves represent the information for the DCMU treated soybean plant.
Figure 61: Minimum and Mean Differences Between Indexes from a Healthy and DCMU Treated Soybean Plant Using a 300 meter Standoff (Small Soil Reflectivity Range)

The red curve represents the minimum separation necessary to distinguish the two conditions. The blue curve is the actual separation in index value between the two conditions.
Figure 62: Index and Range for a Healthy and DCMU Treated Soybean
Determined at 300 meters with a Background Soil with a Medium Reflectivity Range

Index, range, and standard deviation for a healthy and DCMU stressed soybean plant with a chlorophyll concentration of 40 μg/cm². The blue curves represent the information for a healthy soybean plant. The red curves represent the information for the DCMU treated soybean plant.
The red curve represents the minimum separation necessary to distinguish the two conditions. The blue curve is the actual separation in index value between the two conditions.

The index signals from soybean plants at different chlorophyll concentrations are undergoing the same trends mentioned for the DCMU stress at this distance. There is much overlap in the index ranges at lower LAI values (especially between 10 and 20 µg/cm² of chlorophyll). Figure 64 shows how the indexes and ranges relate to one another for the different chlorophyll levels. The index ranges for 10 and 20 µg/cm² both overlap across all LAI values. The index ranges for 20 and 40 µg/cm² are significantly separated except at low LAI values. When comparing the 40 and 60 µg/cm² chlorophyll conditions the index ranges slightly overlap at all LAI values.

What this translates into is situations where the different chlorophyll levels are not perceptible from one another. For low LAI values none of the different chlorophyll levels can be separated (see Figure 65). How high the LAI index must be before separation is possible depends on what two chlorophyll levels are being compared.

Figure 65 shows that the difference between 20 and 40 µg/cm² has the biggest gap between the actual index difference and the minimum separation needed for detection. This is followed by the separation between 40 and 60 µg/cm² with the
separation between 10 and 20 $\mu g/cm^2$ being the smallest. The minimum LAI needed to detect a difference increases as one goes from the first to last comparison mentioned above.

In order to determine the difference between the different chlorophyll levels, at this standoff, a minimum level of canopy LAI has to be reached. As mentioned above this depends on the conditions compared. When looking at all of the chlorophyll cases a minimum LAI of 2.3 (for a medium range soil reflectivity) needs to be present in order for all conditions to be separated from one another. The reason is a LAI of at least 2.3 is needed when trying to distinguishing the 10 $\mu g/cm^2$ and 20 $\mu g/cm^2$ chlorophyll levels. At higher LAI levels the difference between indexes at 10 $\mu g/cm^2$ and 20 $\mu g/cm^2$ is just large enough to be distinguished from one another. The signal from these two conditions pushes the ability of the system to see a difference at this point.

In contrast to this, the index values between a chlorophyll level of 20 $\mu g/cm^2$ and 40 $\mu g/cm^2$ are clearly separated. Therefore some conditions are more readily distinguishable from one another than others. Taking this into consideration one has to account for the weakest signal when making an assessment of a system. In this case a minimum LAI of 2.3 would be needed for each chlorophyll level to be seen from one another.
Figure 64: Index and Ranges for Different Soybean Chlorophyll Levels and Two Different Soil Reflectivity Ranges Determined for a 300 meter Standoff

The red curves are the index and range for a soybean plant with a chlorophyll concentration of 10 μg/cm². The blue curves are the index and range for a soybean plant with a chlorophyll concentration of 20 μg/cm². The green curves are the index and range for a soybean plant with a chlorophyll concentration of 40 μg/cm². The magenta curves are the index and range for a soybean plant with a chlorophyll concentration of 60 μg/cm².
Figure 65: Minimum and Actual Differences between Indexes for Different Chlorophyll Levels of Soybean Determined for a 300 meter Standoff

Plots for a small range in soil reflectivity are listed on the left. Plots for a medium range in soil reflectivity are listed on the right. The red curve represents the minimum separation necessary to distinguish the two conditions. The blue curve is the actual separation in index value between the two conditions.
In order to reliably distinguish between the six soybean plant conditions at this level a minimum LAI of 2.3 is needed. If this is the case one can distinguish between all six soybean conditions mentioned in this research.

It appears as long as one is measuring a soybean canopy with a LAI of two or more, many different canopy conditions can be discriminated (utilizing pixel averaging). This is a reasonable expectation given that a mature soybean canopy (older than a month) has a LAI of at least two. Another benefit is the variation in the fluorescent ratio is small across LAI values higher than two.

7. Conclusions

The following is a discussion on the conclusions made in this research. It is comprised of three parts. The first part represents the remarks made on the results. The second involves recommendations for actual field collections. The third discusses areas of future research that would be beneficial for this area of interest.

The results for determining whether a change in plant condition could be seen by a change in index value was based on a specific measurement scenario. That scenario involved the measurement of a soybean plant (with a spherical type canopy) using three different sensor platforms (at three different standoff distances: 10, 30, 300 meters). The soybean plants were also considered to have six different states of condition (before and after DCMU treatments, 4 different levels of chlorophyll concentration: 10, 20, 40, 60 μg/cm²). The plants were considered to be measured on a mid summer day at 5 P.M. in the afternoon in the state of Georgia. The collection was assumed to take place from a nadir point of view utilizing an excitation wavelength of 632.8 nm. Using this scenario the fluorescent signal was calculated for such a situation.

The results show that the signal to noise ratio is high enough to allow the detection of the fluorescent signal at all three sensor platforms. However they also show (when measured with one resolution element) that they are too low allow the detection of
different plant conditions. Therefore pixel averaging over the Xybion camera had to be utilized to effectively reduce the noise. The sensor systems at 10, 30, and 300 meter standoff distances were averaged over 4, 4, and 16 resolution elements respectively. By combining the signal from adjacent camera resolution elements the SNR is effectively enhanced. The draw back being the spatial resolution of the camera is effectively reduced. Therefore when dealing with systems measuring at these distances a decision has to made on output. In order to distinguish plant conditions you either have a lower standoff distance or utilize a lower spatial resolution.

The results also show that the laser excitation power may not be large enough to produce a fluorescent signal that is detectable at distances significantly larger than 300 meters. A larger sensor system standoff would require a higher power laser. The sensor signal to noise ratio could also be increased by averaging over more detector resolution elements. This would increase the recorded signal at the sacrifice of recorded spatial resolution. This could possibly allow the measurement of plant condition at further standoff distances.

The resulting analysis of the fluorescent ratio 690 nm/ 730 nm shows that the difference between certain plant conditions can be seen. In the case of the hand held sensor system (with a 10 meter standoff and averaging over 4 resolution elements) the differences in the ratio were clearly evident between two separate plant conditions as long as the LAI was above one. The index values were able to resolve the difference between a healthy and DCMU treated soybean. It was also able to determine the difference between all four chlorophyll concentrations. It was able to resolve all six conditions as long as the canopy density had a LAI above one.

Using a field based collection system, with a 30 meter standoff (and averaging over 4 resolution elements) the results were equally favorable. Even though the index values begin to become closer in scope, the overall differences between plant conditions were still evident. Except for instances of canopy leaf area index below 1.5, all six
conditions could be distinguished. The index value illustrated the difference between a DCMU treated and untreated plant. It also showed the differences between soybean plants with different levels of chlorophyll.

When using an air based sensor system with a 300 meter standoff (and averaging over 16 resolution elements) less favorable results occurred. While the difference between all six conditions was still evident, it was only true for soybean canopies with a leaf area index greater than 2.3. Even with a LAI of greater than 2.3 some conditions were just separable. In the case of DCMU stress and the separation of chlorophyll levels between 10 and 20 μg/cm² and between 40 and 60 μg/cm² the difference in index was just larger than the minimum needed to see the separation in values (at a 85% confidence level). Only between the chlorophyll levels of 20 and 40 μg/cm² was the separation in index values much larger than the necessary minimum.

At canopies containing a LAI of much less than two only two conditions could be separated. The two chlorophyll levels of 20 to 40 μg/cm² and 40 to 60 μg/cm² could be discerned for LAI values lower than two. However, when the LAI became too low these condition were indistinguishable also. Therefore in order to be able to distinguish all six conditions, at a standoff of 300 meters, the soybean canopy had to have a leaf area index of at least 2.3.

The result of these six stress conditions should be applicable to other stress conditions. The plant conditions chosen included the inhibition of the photosynthetic process and changing chlorophyll content. Both situations occur for different types of stress. Therefore the results determined here should be comparable to the fluorescent indexes of a plant under different stress conditions.

Over the course of this research there were many factors identified that alter the fluorescent ratio 690 nm/ 730 nm. Many of these factors were isolated due to the scenario chosen. One thing to keep in mind is when one begins to think about these results being applied to a different scenario all these factors need to be included in the
process. Such issues as ambient light level, collection view, canopy type, and laser excitation wavelength need to be considered.

The results from this research indicate that leaf area index, canopy type, soil reflectance, amount of fluorescence from the inner canopy, atmospheric factors, and sensor system characteristics all affect the remotely measured index value of a plant. One of the largest variables in the resulting index value being the leaf area index of the plant. There is a large drop in the index value from small LAI values to larger ones. This is important when trying to identify the expected index value from a plant. A simple change in canopy density can alter the measured index. Therefore to provide for measurements that provide more comparable results, the measurement of plant canopy fluorescence should be accompanied by a leaf area index measurement. One way the LAI can be determined remotely is with a reflectance measurement [Castagnoli et al., 1988]. This would be especially important during early growing stages where the plants have a low leaf area index. When the plant has matured (grown more than a month) it has been shown that the leaf area index is above two [Savoy et al., 1992]. Therefore making a LAI assessment is not as vital. For LAI above two the index value is more consistent across an increasingly dense canopy. Therefore fluorescent measurement of young plants provides more of a challenge than of a mature plant set. The problem here being with a cash crop one most likely has more interest in crop stress early on in the plants development. Later in maturity one may not be able to rectify the stress conditions a plant has undergone.

Another measurement issue is what index value (690 nm/730 nm ratio) to expect for a situation. The effects of the canopy, atmosphere, and sensor system have a clear affect on the measured index value. As was mentioned in the results the canopy and atmosphere both cause the ratio to drop from the leaf level fluorescent ratio. The camera responsivity of the sensor system is weighted towards energy at the 690 nm band so it dramatically raises the ratio. If this is not taken into account a healthy ratio at the leaf
level (ex. 0.8) may be taken as a stressed plant when the index is measured remotely (ex. 1.2). Without adjusting for this factor the plant fluorescence may be misinterpreted. However being knowledgeable about this situation will make the separation between plant conditions possible. The fact that a remotely measured soybean plant will take on a higher initial index value for a healthy plant needs to be realized.

One way to change this dramatic change in ratio values would be to utilize a camera with a response curve that is more uniform throughout the range of wavelengths in bands 690 nm and 730 nm. One could also incorporate the camera sensor response in post analysis of the measured fluorescent bands. In doing this the differences in responsivity could be accounted for. Therefore after post processing the index would be representative of an index from a camera with a flat response. At this point the canopy and atmosphere would be the largest factors in altering the index ratio. The resulting indexes would be closer to the original leaf level measurement.

The fact that this research was modeling a spherical type canopy means the index value was more susceptible to inner canopy and background effects. This is due to a spherical canopy having a leaf inclination that lends itself more transparent to incoming light. Therefore less light is attenuated by the top layers. Both uniform and planophile canopy types have a leaf inclination that attenuates more light at the top layers. This causes the index value from the canopy to typically have less variability. Therefore as long as the plant canopy under analysis does not have a leaf inclination angle larger than a spherical canopy the results should not have any larger a range in index than presented here.

Another issue in making a field collection is the amount of ambient light. In this research the effects of this variable were reduced by assuming all measurements were taken at the same time of day (same ambient light level). In order to repeat this with an actual collection it is recommended that an ambient light level measurement be taken along with the fluorescent measurement. In this way the amount of ambient light can be
verified to be the same during different collections. The time of day is not the only factor influencing the amount of light. The day of the year, clouds, and atmosphere conditions all affect the amount of ambient light reaching a plant. By measuring the ambient light level, collections made under very different conditions can be made if the level is similar. In taking this measurement one may also begin to further examine the effects different ambient light levels have on the plant’s fluorescent output.

There are several areas that need to be looked into to further improve the results of this research. First off, better quantum efficiency values need to be determined. In this research a corn leaf plant (excited at 532 nm) was assumed to have the same quantum efficiencies as a soybean plant (excited at 632.8 nm). This is not a favorable assumption to make. In future efforts it would benefit analysis greatly if the actual quantum efficiencies of the plant in question were determined for the excitation wavelength being used. Knowing the quantum efficiencies of the plant for different plant conditions would even further enhance the modeling of these signals at a remote location.

Another issue was the modeled excitation wavelength. The excitation wavelength used in the calculations was chosen because of its widespread use in past fluorescent research. It was also chosen due to the attenuation coefficients for this wavelength were present in the canopy model. The problem is this is not the ideal wavelength for plant fluorescence. It would be interesting and beneficial to find the expected index values at a remote location for different excitation wavelengths. This would warrant identifying the proper input parameters for the canopy model at different wavelengths. This type of research would benefit the ongoing work done in the field. The newest research in the laser induced fluorescent field is starting to address how a plant’s fluorescent spectrum changes due to the chosen wavelength of excitation. The ability to model the expected index value from a remote platform using a certain wavelength laser would be desirable.
Another issue is the change in chlorophyll level. When viewing the changes in index at different chlorophyll levels, a certain change in chlorophyll is not always accompanied by an equal change in the index value. This can be seen between the chlorophyll level 20 μg/cm² and 40 μg/cm² and between 40 μg/cm² and 60 μg/cm². While both have a chlorophyll difference of 20 μg/cm² their respective changes in index are not the same. It would be of interest to determine how small an incremental change in chlorophyll can be detected and how it changes over the range of chlorophyll concentrations for a plant. This would provide a map for plant deterioration and how much deterioration is needed to be detected by a remote sensor platform. In this way one might be able to determine if a certain type of stress can be detected early enough (from a particular sensor platform) to be rectified before permanent damage is done on the plant. Monitoring a widespread area that only allows permanent plant damage to be recorded is less beneficial to a cash crop farmer. This would rule out prevention and could only be used in crop loss analysis. In this way one could determine what type of fluorescent system would be necessary to monitor their crops.

The canopy model utilized in this research could also be enhanced. One item it does not include is multiple scattering within the canopy. While this is most likely a small factor it is a canopy condition that may affect the fluorescent signal. A more realistic canopy model could be formed by including it’s affects. Another improvement would be to identify the interception and attenuation coefficients needed to model plant canopies of other species. This would allow comparison of fluorescent signatures from different plant types. The model also assumes that the leaf layers are homogeneous meaning there is no variation in leaf optical properties and leaf angle distribution. The benefit of this was it simplified the computation process. On the down side this is not true of real plant canopies. A better model would include this factor.
The above research supports the hypothesis that stimulated fluorescent emission, from a plant canopy, can be remotely measured in the form of band ratios to indicate plant condition.
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9. **Appendix**

9.1 **Control Input Data**

**ENTER FILE NAME FOR PRODUCED CARD DECK:**

```
card.dat
```

***** CARD 1 ***

**INPUT ATMOSPHERIC MODEL TYPE**

```
MODEL= 0 IF MESEOROLOGICAL DATA ARE SPECIFIED (HORIZONTAL PATH ONLY)
1 TROPICAL ATMOSPHERE
2 MID-LATITUDE SUMMER
3 MID-LATITUDE WINTER
4 SUB-ARCTIC SUMMER
5 SUB-ARCTIC WINTER
6 1976 U.S. STANDARD ATMOSPHERE
7 NEW MODEL ATMOSPHERE (RADIOSONDE DATA)
```

**CHOOSE A MODEL NUMBER:**

```
2
```

**INPUT THE TYPE OF ATMOSPHERIC PATH**

```
ITYPE= 1 FOR A HORIZONTAL (CONSTANT PRESSURE) PATH
2 VERTICAL OR SLANT PATH BETWEEN TWO ALTITUDES
3 VERTICAL OR SLANT PATH TO SPACE
```

**CHOOSE A TYPE:**

```
2
```

**PROGRAM EXECUTION MODE**

```
IEMSCT= 0 PROGRAM EXECUTION IN TRANSMITTANCE MODE
1 PROGRAM EXECUTION IN RADIANCE MODE
2 PROGRAM EXECUTION IN RADIANCE MODE
   WITH SOLAR/LUNAR SCATTERED RADIANCE INCLUDED
3 DIRECT SOLAR IRRADIANCE
```

**ENTER EXECUTION MODE:**

```
2
```

**MULTIPLE SCATTERING EXECUTION MODE**

```
IMULT= 0 PROGRAM EXECUTED W/OUT MULTIPLE SCATTERING
1 PROGRAM EXECUTED WITH MULTIPLE SCATTERING
```

**MULTIPLE SCATTERING MODE:**

```
1
```

**DO YOU WANT TO MODIFY THE DEFAULT ALTITUDE PROFILES OF TEMPERATURE AND PRESSURE (Y OR N)?**

```
n
```

**DO YOU WANT TO MODIFY THE DEFAULT ALTITUDE PROFILE OF WATER VAPOR (Y OR N)?**

```
n
```

**DO YOU WANT TO MODIFY THE DEFAULT ALTITUDE PROFILES OF OZONE (Y OR N)?**

```
n
```

**DO YOU WANT TO MODIFY THE DEFAULT SEASONAL DEPENDENCE OF CH4 (Y OR N)?**

```
n
```

**DO YOU WANT TO MODIFY THE DEFAULT SEASONAL DEPENDENCE OF N2O (Y OR N)?**

```
n
```
Do you want to modify the default seasonal dependence of CO (Y or N)?

Do you want to print the atmospheric profiles (Y or N)?

What is the temperature of the Earth (boundary layer) in degrees K (0.0 uses the first radiosonde reading)? 300.0

Enter the surface albedo (0.00 is a blackbody) 1.0

*** Card 2 ***

Select an aerosol extinction

IRAZE= 0 NO AEROSOL ATTENUATION INCLUDED IN CALCULATION
1 RURAL EXTINCTION, 23-KM VIS.
2 RURAL EXTINCTION, 5-KM VIS.
3 NAVY MARITIME EXTINCTION, SETS OWN VIS.
4 MARITIME EXTINCTION, 23-KM VIS.
5 URBAN EXTINCTION, 5-KM VIS.
6 TROPOSPHERIC EXTINCTION, 50-KM VIS.
7 USER DEFINED AEROSOL EXTINCTION COEFFICIENTS TRIGGERS READING IREG FOR UP TO 4 REGIONS OF USER DEFINED EXTINCTION ABSORPTION AND ASYMMETRY
8 ADVECTION FOG EXTINCTION, 0.2-KM VIS.
9 RADIATION FOG EXTINCTION, 0.5-KM VIS.
10 DESERT EXTINCTION SETS OWN VISIBILITY FROM WIND SPEED

Choose aerosol extinction type:
1

Select a season

ISEASN= 0 DEFAULT SEASON FOR MODEL
(SUMMER FOR MODELS 0,1,2,4,6,7)
(WINTER FOR MODELS 3,5)
1 SPRING-SUMMER
2 FALL-WINTER

Choose a season:
0

Select a volcanic aerosol extinction

IVULCN= 0 DEFAULT TO STRATOSPHERIC BACKGROUND
1 STRATOSPHERIC BACKGROUND
2 AGED VOLCANIC TYPE/MODERATE VOLCANIC PROFILE
3 FRESH VOLCANIC TYPE/HIGH VOLCANIC PROFILE
4 AGED VOLCANIC TYPE/HIGH VOLCANIC PROFILE
5 FRESH VOLCANIC TYPE/MODERATE VOLCANIC PROFILE
6 BACKGROUND STRATOSPHERIC TYPE/MODERATE VOLCANIC PROFILE
7 BACKGROUND STRATOSPHERIC TYPE/HIGH VOLCANIC PROFILE
8 FRESH VOLCANIC TYPE/EXTREME VOLCANIC PROFILE
CHOOSE A VOLCANIC EXTINCTION:
0

SPECIFY CLOUD/RAIN RATE MODEL
ICLD 0 NO CLOUDS OR RAIN
1 CUMULUS CLOUD BASE .66KM TOP 2.7KM
2 ALTOSTRATUS CLOUD BASE 2.4KM TOP 3.0KM
3 STRATUS CLOUD BASE .33KM TOP 1.0KM
4 STRATUS/STRATOCUMULUS CLOUD BASE .66KM TOP 2.0KM
5 NIMBOSTRATUS CLOUD BASE .16KM TOP .66KM
6 2.0MM/HR DRIZZLE (MODELED WITH CLOUD 3)
   RAIN 2.0MM/HR AT 0KM TO .22MM/HR AT 1.5KM
7 5.0MM/HR LIGHT RAIN (MODELED WITH CLOUD 5)
   RAIN 5.0MM/HR AT 0KM TO .2MM/HR AT 1.5KM
8 12.5MM/HR MODERATE RAIN (MODELED WITH CLOUD 5)
   RAIN 12.5MM/HR AT 0KM TO .2MM/HR AT 2.0KM
9 25.0MM/HR HEAVY RAIN (MODELED WITH CLOUD 1)
   RAIN 25.0MM/HR AT 0KM TO .2MM/HR AT 3.0KM
10 75.0MM/HR EXTREME RAIN (MODELED WITH CLOUD 1)
   RAIN 75.0MM/HR AT 0KM TO .2MM/HR AT 3.5KM
11 USER DEFINED CLOUD EXTINCTION, ABSORPTION, AND AEROSOL
   EXT. COEFFICIENTS' TRIGGERS READING IREG FOR UP TO 4
   REGIONS OF EXTINCTION ABSORPTION + ASYMMETRY
18 STANDARD CIRRUS MODEL
19 SUB VISUAL CIRRUS MODEL
20 NOAA CIRRUS MODEL (LOWTRAN6)

CHOOSE A CLOUD MODEL:
0

DO YOU WANT TO USE ARMY VERTICAL STRUCTURE ALGORITHM FOR AEROSOLS
IN BOUNDARY LAYER (Y OR N)?
n
DO YOU WANT TO OVERRIDE THE DEFAULT VISIBILITY (Y OR N)?
n
WHAT IS THE RAIN RATE? (MM/HR)
0.0

WHAT IS THE GROUND ALTITUDE ABOVE SEA LEVEL? (KM)
0.218

*** CARD 3 ***

ENTER H1, INITIAL ALTITUDE (KM) (OBSERVER POSITION):
0.518

ENTER H2, FINAL ALTITUDE (KM):
0.218

ENTER INITIAL ZENITH ANGLE (DEGREES) AS MEASURED FROM INITIAL ALTITUDE
(NOTE: 0 LOOKS STRAIGHT UP, 180 STRAIGHT DOWN):
180.0

ENTER PATH (RANGE) LENGTH (KM):
0.0

ENTER EARTH CENTER ANGLE SUBTENDED BY H1 AND H2 (DEGREES): 0.0

DO YOU WANT TO OVERRIDE THE DEFAULT EARTH RADIUS (Y OR N)? n

USE THE SHORT PATH FROM OBSERVER'S TO FINAL ALTITUDE (Y OR N)? y

*** CARD 3A1 ***

SPECIFY THE GEOMETRY OF THE OBSERVATION

IPARM 0 SPECIFY 1 OBSERVER LATITUDE
       2 OBSERVER LONGITUDE
       3 SOURCE LATITUDE
       4 SOURCE LONGITUDE

IPARM 1 SPECIFY 1 OBSERVER LATITUDE
       2 OBSERVER LONGITUDE

IPARM = 2 SPECIFY 1 AZIMUTHAL ANGEL
       2 ZENITH ANGLE OF THE SUN

CHOOSE A TYPE OF GEOMETRY SPECIFICATION:
1

IPH 0 HENGYEY-GREENSTEIN AEROSOL PHASE FUNCTION
     1 USER SUPPLIED AEROSOL PHASE FUNCTION
     2 MIE GENERATED DATA BASE OF AEROSOL PHASE
        FUNCTIONS FOR THE LOWTRAN MODELS

ENTER PHASE FUNCTION TYPE: 2

ENTER THE DAY OF THE YEAR (I.E. FROM 1 TO 365): 182

*** CARD 3A2 ***

ENTER OBSERVER LATITUDE (-90 TO 90): 34

ENTER OBSERVER LONGITUDE (0 TO 360): 86

ENTER TIME OF DAY IN DECIMAL HOURS: 21.0

ENTER PATH AZIMUTH AS DEGREES EAST OF NORTH: 0.0

*** CARD 4 ***

WHAT UNITS ARE YOU USING FOR WAVELENGTH? (MICRONS OR NANOMETERS)
microns

INPUT STARTING AND ENDING WAVELENGTH ON BANDPASS
0.4

209
HOW MANY INTERVALS ACROSS BANDPASS? (MAXIMUM 396) 396

*** CARD 5 ***

IRPT= 0 TO END LOWTRAN 6 RUN
      1 TO READ ALL DATA CARDS AGAIN
      3 TO READ ONLY CARD 3 AGAIN (GEOMETRY DATA)
      4 TO READ ONLY CARD 4 AGAIN (WAVELENGTH RANGE)

SELECT IRPT:
0
### 9.2 Lowtran Card Deck for 10 Meters

<p>| 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 0 | 0 | 300.000 | 1.00 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| -99.000 | -99.000 | -99.000 | -99.000 | -99.000 | -99.000 | 33MIDLATITUDE SUMMER |
| 0.228 | 0.218 | 180.000 | 0.010 | 0.000 | 0.000 | 0 |
| 1 | 2 | 182 | 0 | 34.000 | 86.000 | 23.170 | 134.120 | 21.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 12500.000 | 25000.000 | 30.000 |
| -9999. |
| 2 | 3 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 0 | 0 | 300.000 | 1.00 |
| 1 | 1 | 1 | 1 | 0 | 0 | 23.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.218 |
| -99.000 | -99.000 | -99.000 | -99.000 | -99.000 | -99.000 | 33MIDLATITUDE SUMMER |
| 0.218 | 100.000 | 85.000 | 714.080 | 6.312 | 0.000 | 0 |
| 1 | 2 | 182 | 0 | 34.000 | 86.000 | 23.170 | 134.120 | 21.000 | -148.068 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 12500.000 | 25000.000 | 30.000 |
| -9999. |
| 2 | 3 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 0 | 0 | 300.000 | 1.00 |
| 1 | 1 | 1 | 1 | 0 | 0 | 23.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.218 |
| -99.000 | -99.000 | -99.000 | -99.000 | -99.000 | -99.000 | 33MIDLATITUDE SUMMER |
| 0.218 | 100.000 | 75.000 | 352.116 | 3.013 | 0.000 | 0 |
| 1 | 2 | 182 | 0 | 34.000 | 86.000 | 23.170 | 134.120 | 21.000 | -148.068 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 12500.000 | 25000.000 | 30.000 |
| -9999. |
| 2 | 3 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 0 | 0 | 300.000 | 1.00 |
| 1 | 1 | 1 | 1 | 0 | 0 | 23.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.218 |
| -99.000 | -99.000 | -99.000 | -99.000 | -99.000 | -99.000 | 33MIDLATITUDE SUMMER |
| 0.218 | 100.000 | 60.000 | 195.283 | 1.498 | 0.000 | 0 |
| 1 | 2 | 182 | 0 | 34.000 | 86.000 | 23.170 | 134.120 | 21.000 | -148.068 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 12500.000 | 25000.000 | 30.000 |
| -9999. |</p>
<table>
<thead>
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<tbody>
<tr>
<td>0.218</td>
<td>100.000</td>
<td>45.000</td>
<td>140.074</td>
<td>0.877</td>
<td>0.000</td>
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33MIDLATITUDE SUMMER

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