AN ULTRAVIOLET LIGHT EMITTING DIODE LED-INDUCED CHLOROPHYLL FLUORESCENCE IMAGING SYSTEM FOR MONITORING FRUITS: FROM BENCH TO COMMUNITY

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GHANA ACADEMY OF ARTS AND SCIENCES


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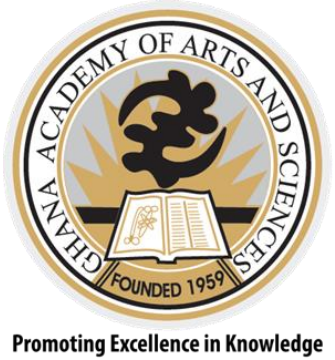

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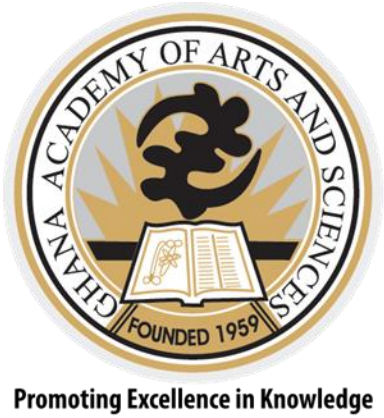


FRUITS

The bench and the idea

Assembling components

Measuring parameters

Testing on products and Applications

Transfer to Community

Conclusion

Imagine you could pop a scanner from your handbag to see precisely how ripe the bananas are that you want to buy, or to ascertain how long you can still store them before they need to be used in a fruit salad?

KNOWING WHEN IT'S TIME TO

eat your fruit

By Engela Duvenage

A nifty gadget, don't you think? And not at all too farfetched, it seems.

Detection systems have been developed to detect crop conditions by measuring the levels of chlorophyll and photosynthesis, and ultimately whether fluorescence is emitted when an ultraviolet light shines on a leaf or a fruit.

"As small size excitation sources, fibre probes and miniature-integrated spectrometers have become commercially available, a lot of development has been done in the past decade," explains Prof Hubertus von Bergmann of the Laser Research Institute (LRI) in the Department of Physics.

Together with physicists from Ghana's University of Cape Coast, Prof von Bergmann and LRI colleague Dr Christine Steenkamp recently developed a portable fibre-probe detection system which is already a vast improvement on similar models.

The LRI offers the only outcomes-based university program in laser physics in the country, and is a driving force in the African Laser Centre (ALC), a continent-wide initiative to stimulate laser physics. The LRI hosts regular ALC training courses at Stellenbosch for physicists from many African countries.

A few years ago Prof Von Bergmann, who is also an ALC director, toured African institutions to assess their research needs and capacity. "Quite a few physics groups were working on diagnostic devices that could benefit the horticultural industry," he remembers. "Research on such detection systems is of special interest to African universities where science with a specific agricultural outcome receives preference."

Researchers in this field use compact fluorescence detection systems to study plant growth and post-harvest losses of fruits. It can also detect various dermatological conditions, such as skin cancer or fungal infections.

The valuable contacts Prof Von Bergmann made led to two separate research projects being funded by the ALC and the National Laser Centre.

Prof Hubertus von Bergmann of the Laser Research Institute shows physics masters student Annelie Griesel and PhD student Gibson Nyamud how the improved portable fibre-probe detection system works. (Photo: Engela Duvenage)

During an extended research visit to Stellenbosch by Ghanaian Prof Paul Buah-Bassuah, the group designed a portable fibre-probe ultraviolet light emitting diode (LED)-induced fluorescence detection system. It is more robust, because it uses less optics, is easily operated, and is relatively immune to ambient light. The design is much more stable under harsh field conditions, which is ideal for on-the-spot measurements in storerooms and orchards.

"By using a LED source we save R25 000 and reduce cost to less than 10% for the excitation source, compared to less stable laser diodes (LD)," says SU physicist Prof Hubertus von Bergmann. "This saves almost 50% per instrument."

It is therefore more cost-effective for use within the African agricultural sector and in other less developed countries.

The system has been tested on lemons, bananas, mandarins and ivy plants.

Prof Buah-Bassuah's visit was followed by that of two Tunisian researchers, Dr Najoua Derbel and Ms Jaouhra Cherif from Tunis El Manar University. They used the system to detect the effects of cadmium poisoning in tomato plants.

"It looks very promising for both horticultural and agricultural applications where post-harvest monitoring becomes paramount and the ripening process in the storage and retail process relies on the environmental factors such as room and storage temperature," explains Prof von Bergmann.

"It is also essential to follow growth patterns of various breeding crops till harvest time, and to monitor their growth under specific stress conditions such as a lack of water or nutrients."

The developed prototype is the size of a dictionary. It is coupled with a laptop computer to download and analyse the recorded information. The data is provided to the user in graph format.

It is, however, not yet commercially available.

That, it seems, is not the physicists' baby. "We're into the science, not the packaging," laughs Prof Von Bergmann.

So maybe you'll have to wait just a little bit longer for your own pocket-sized banana-ripeness-meter.

Light- induced processes on Fruit

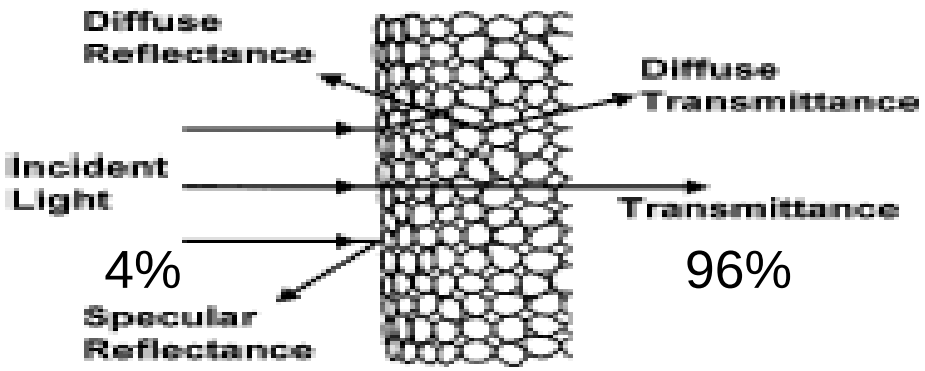


Fig. 1. Incident light on a fruit or vegetable results in specular reflectance (gloss), diffuse reflectance from features at depths to about 5 mm (body relectance or interactance), diffuse transmittance, or absorbance. Color results from very shallow diffuse reflectance.

J.A.Abbott Postharvest Bio and Techn 15 (1999) p207-225

Quality features respond to wavelength in regions outside the visible spectrum. Thus differences in images taken at specific wavelengths from the chlorophyll fluorescence help to distinguish among some physiological events in the fruit as it advances in age. Possible peak wavelengths are 420, 540, 680 ,730 nm

Fluorescence methods for evaluating maturity in fruits is the lose of chlorophyll as they ripen or mature and are sensitive to chilling injury and stress.

Chlorophyll Fluorescence can detect surface damage on fruits in vivo or extract chlorophyll from the peel in vitro for analysis in the near infra red region.

Fluorescence emission bands

Excitation source- UV

Emitted bands:

-440 and 520nm –blue-green fluorescence.

-690 and 740nm –Chlorophyll fluorescence

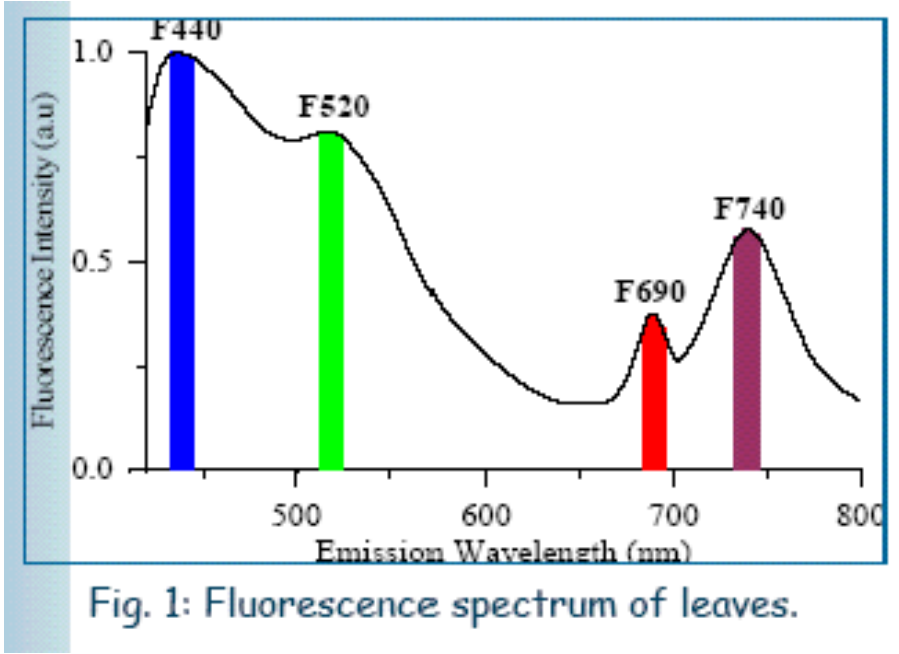
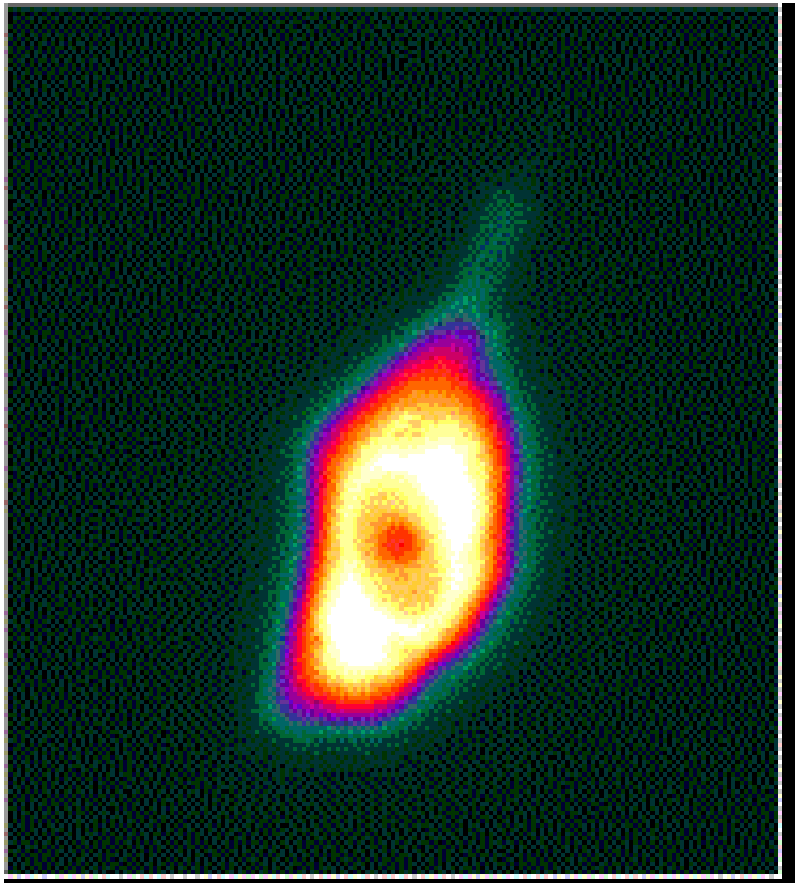


Fig. 1: Fluorescence spectrum of leaves.

Imaging the Fluorescence distribution over leaves can give information on the effects of herbicides, nutrient dificiences, water or heat stresses,etc.

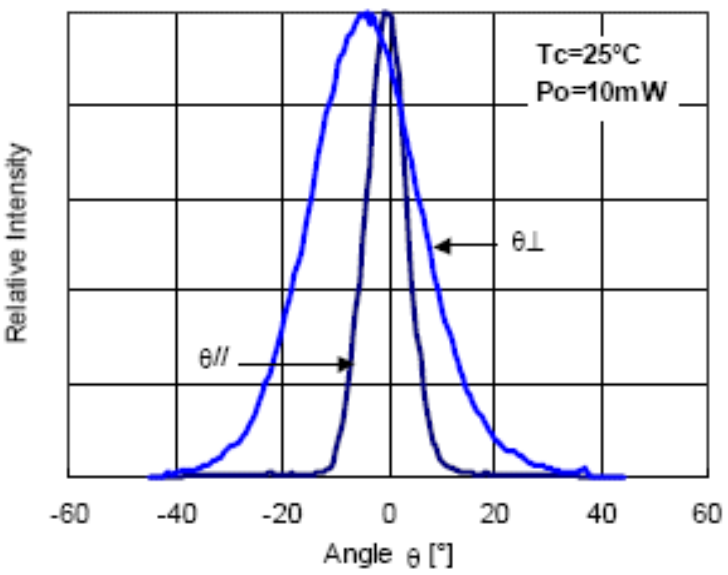
The investigation of vegetation health can be done measuring the fluorescence signals emitted by leaves under UV(355nm) light illumination as in Fig. 1. The emission is studied at 440, 520, 690, and 740nm. Ratio of F440/F690 or F440/F740 and F690/F740 can be used as indicators of plant's health.

Imaging chlorophyll fluorescence of a leaf with different filters

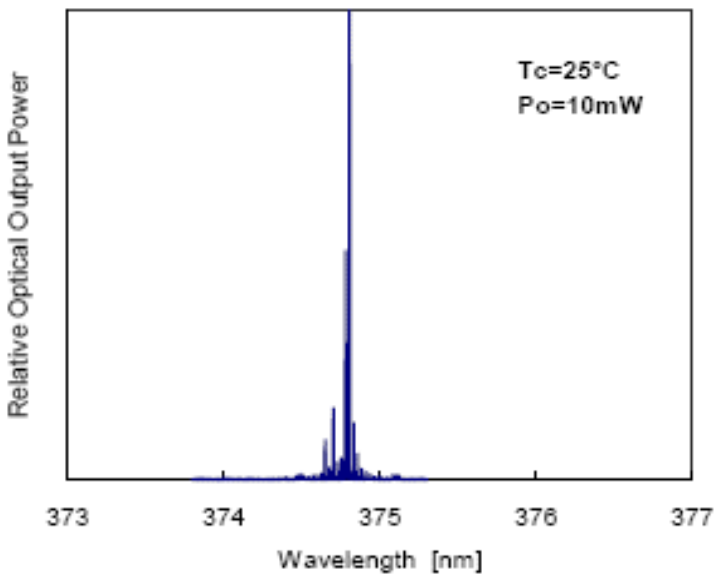


Promoting Excellence in Knowledge

◆Far Field Pattern



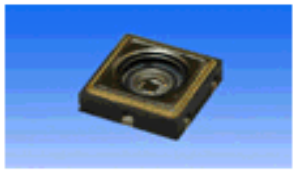
◆Typical Spectrum



NICHIA i-LED

LED characteristics

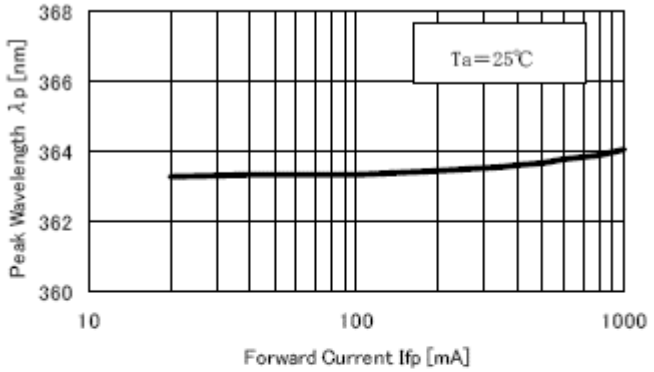
(Tc=25°C / I_F=500mA)

Overview	Size LxWxH(mm)	Product Type	Peak Spectrum λp(nm)		Optical Power Typ (mW)	Forward Voltage V_F (V)		Direction Characteristics 2θ ½ (degree)	Soldering
						Typ	Max		
	6.8x6.8x2.1	NCCU033 P		365	100	4.0	-	100°	Reflow
Applications	U I F S P A								

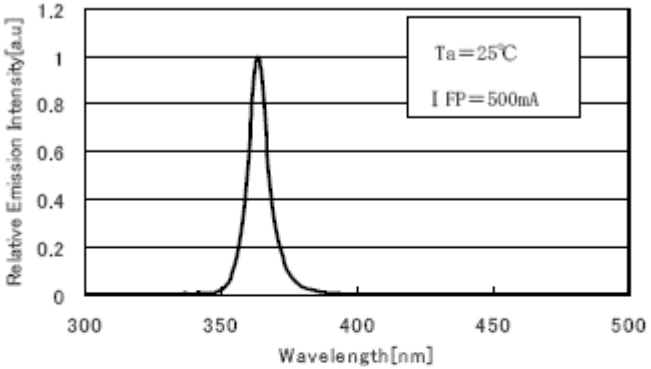
LED Characteristics

I-LED, 100mW, 365nm, 4V

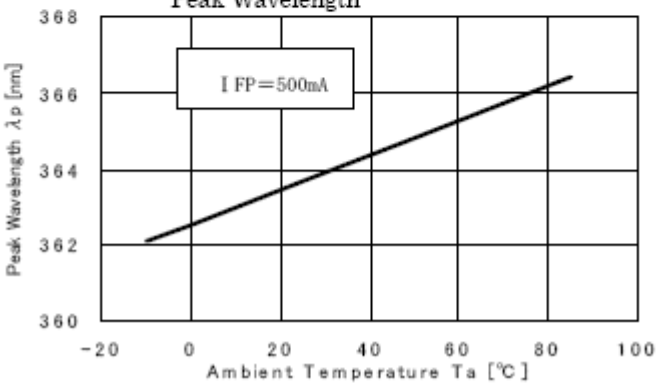
■ Forward Current vs. Peak Wavelength



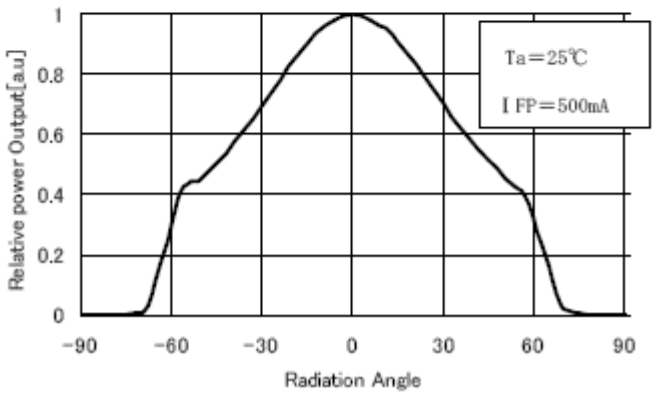
■ Spectrum



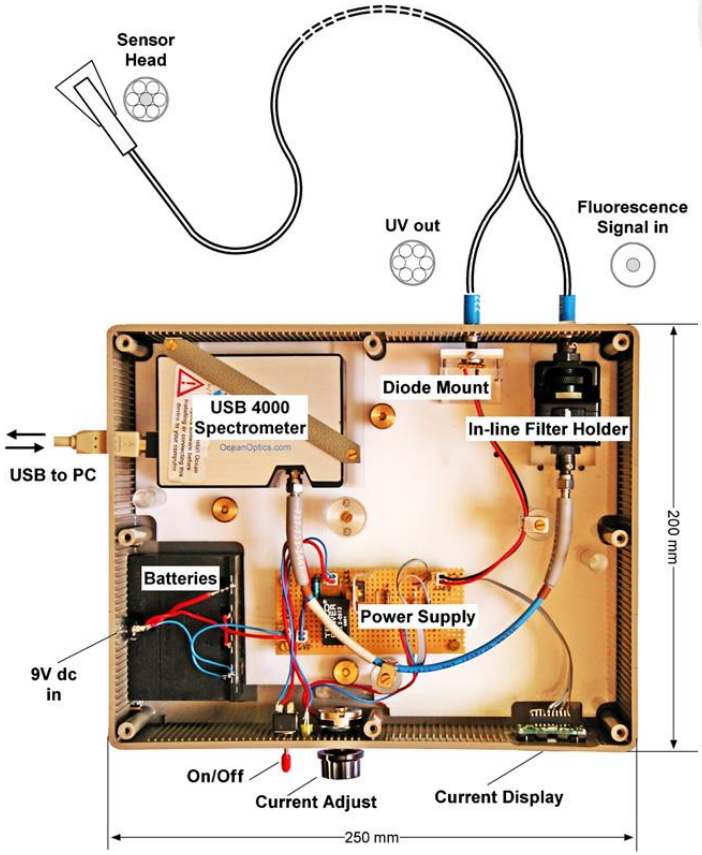
■ Ambient Temperature vs. Peak Wavelength



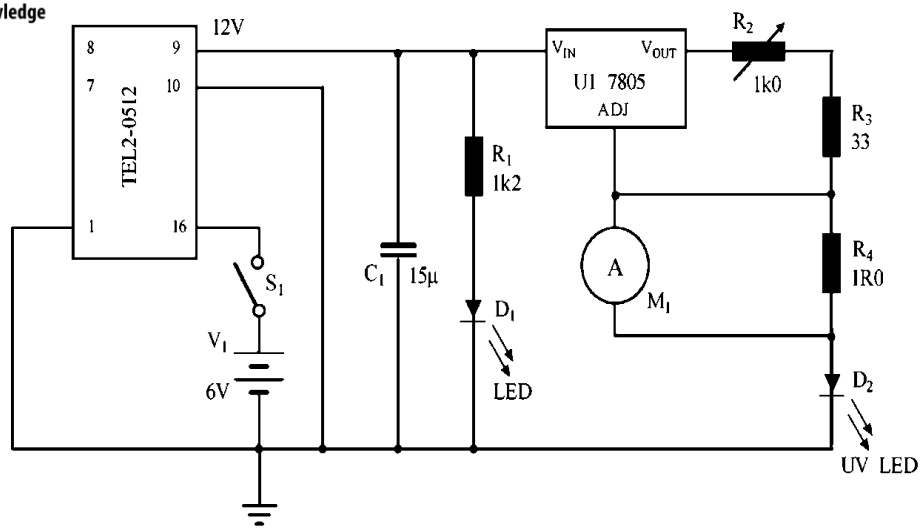
■ Directivity



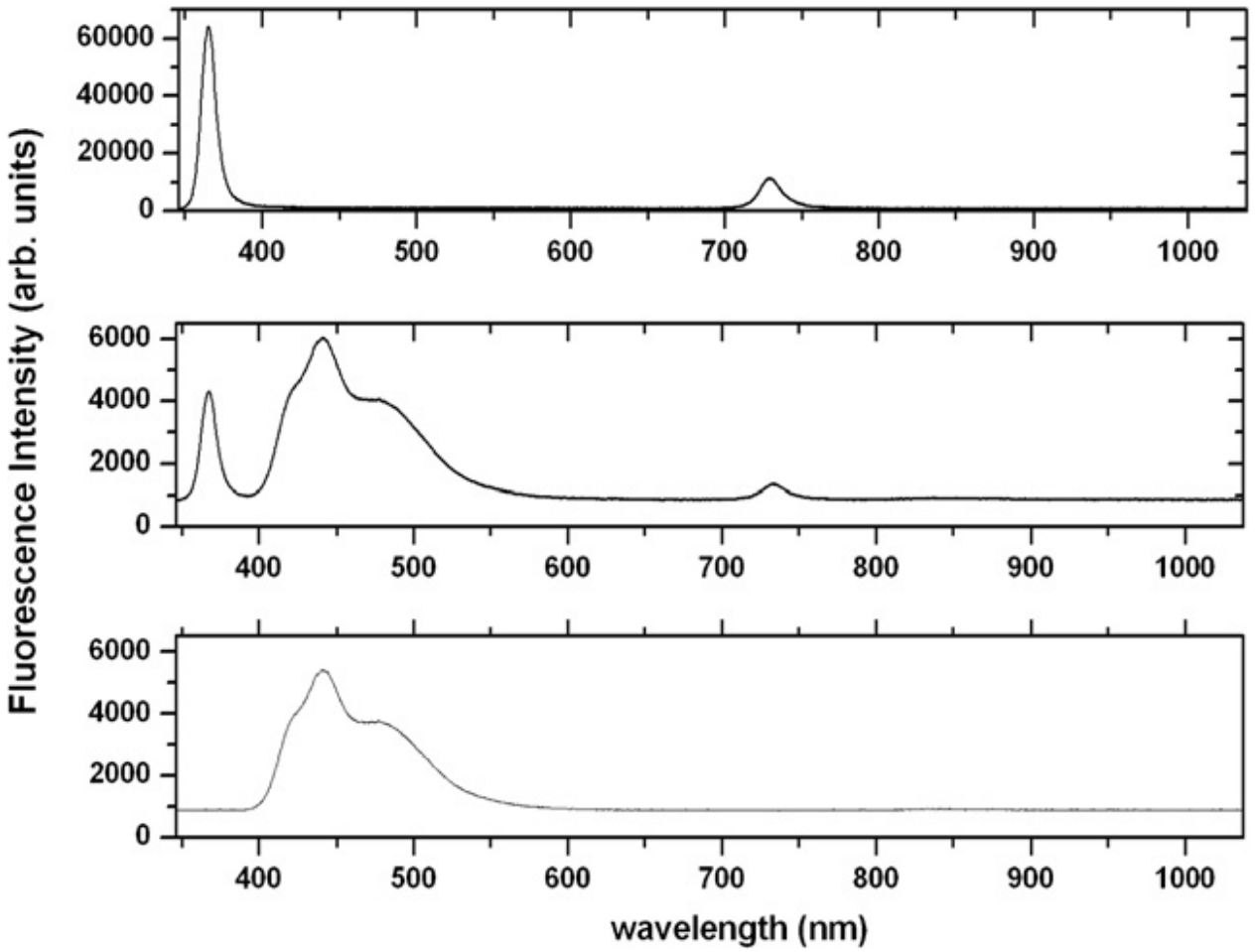
NICHIA CORPORATION	Model	NCCU033
	Title	TYP. CHARACTERISTICS
	No.	050120536821



Layout of the UV LED fibre-probe fluorometer showing a photograph of the assembly of the device with the fibre probe



Circuit diagram for the UV LED driver.



Fluorescence spectra illustrating (*a) the UV LED diode,* (*b) UV LED beam on the white paper target and (c) white paper* using the long-pass filter as a means of explaining the measurement procedure of the system and suppressing the fluorescence from the LED source. There is no fluorescence from the fibre probe.

Quality Assessment.

- **Factors:** Period from harvest to consumer , that is

a) harvest- ripening and maturity

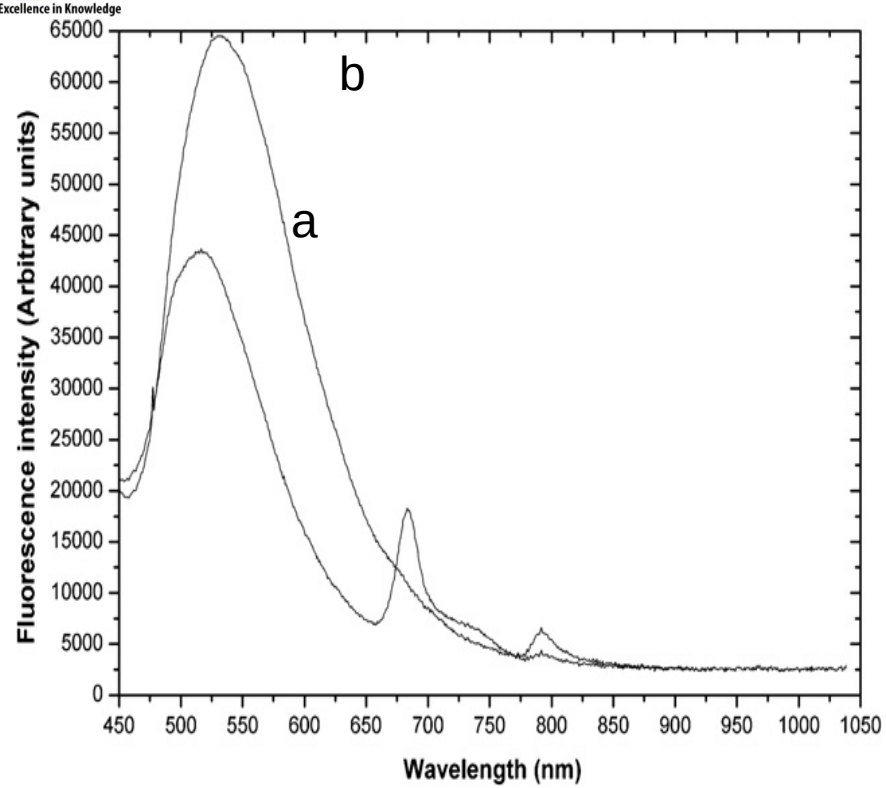
b) handling –chemical or quarantine treatment and storage

c) post-harvest- physiological stages and technological handling

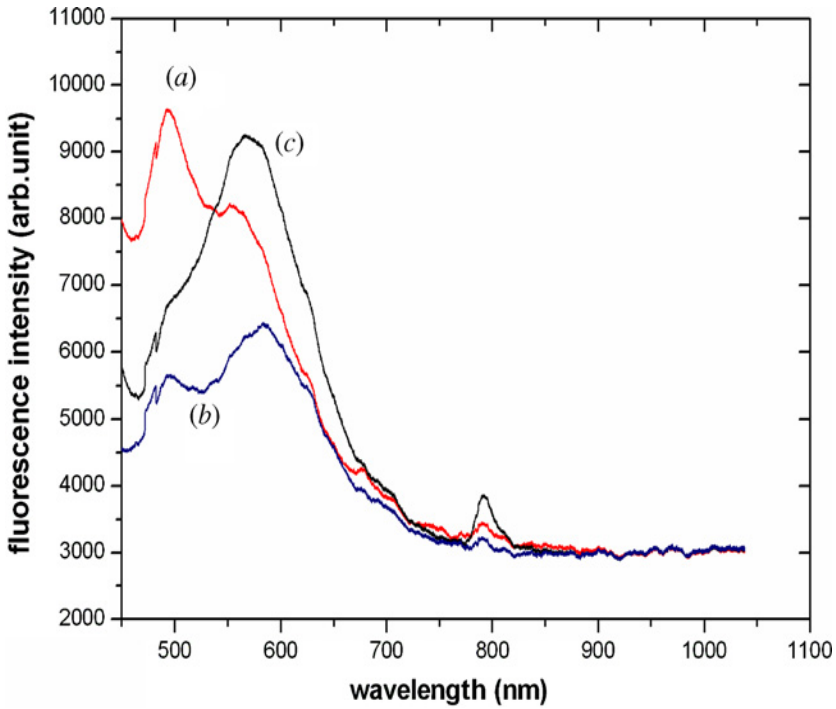
Distinct differences between the spectral signatures of individual fruits are according to different stages of chlorophyll degradation as evident on the different sides of the same fruit..

Chlorophyll Fluorescence Intensity decrease was due to loss in chlorophyll content or photosynthetic activity leading to reduced PSII activity.The fluorescence at 690nm filter is due to PSII and the fluorescence at 730nm is due to PSI.

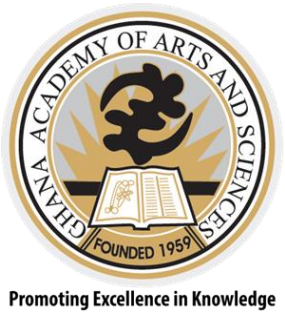
Using a severe chilling process made the fruit loose a large amount of water causing a rapid fluorescence decrease response.



Fluorescence spectra of lemon skin showing
(*a) greenish-yellow skin lemon and*
(b) yellow skin lemon.



Fluorescence spectra of mandarin skin showing (*a) greenish-orange,*
(b) yellowish-orange and
(c) orange skin mandarin.



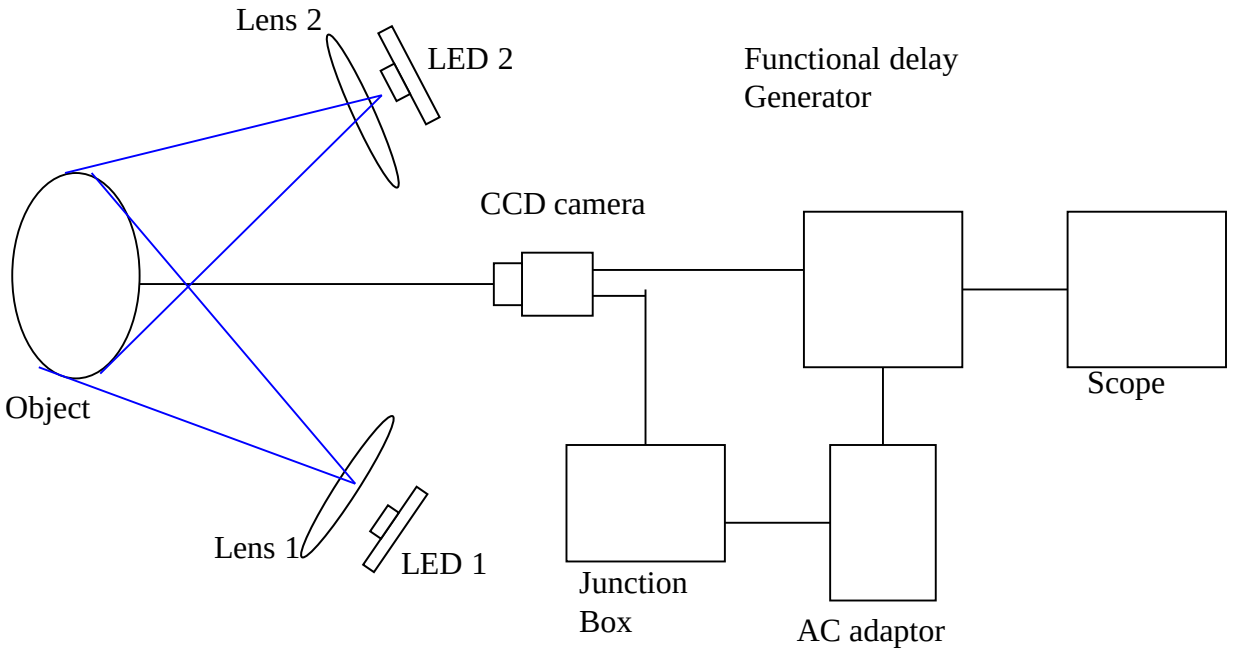
Imaging with He-Ne Laser and UV-LED on fruits

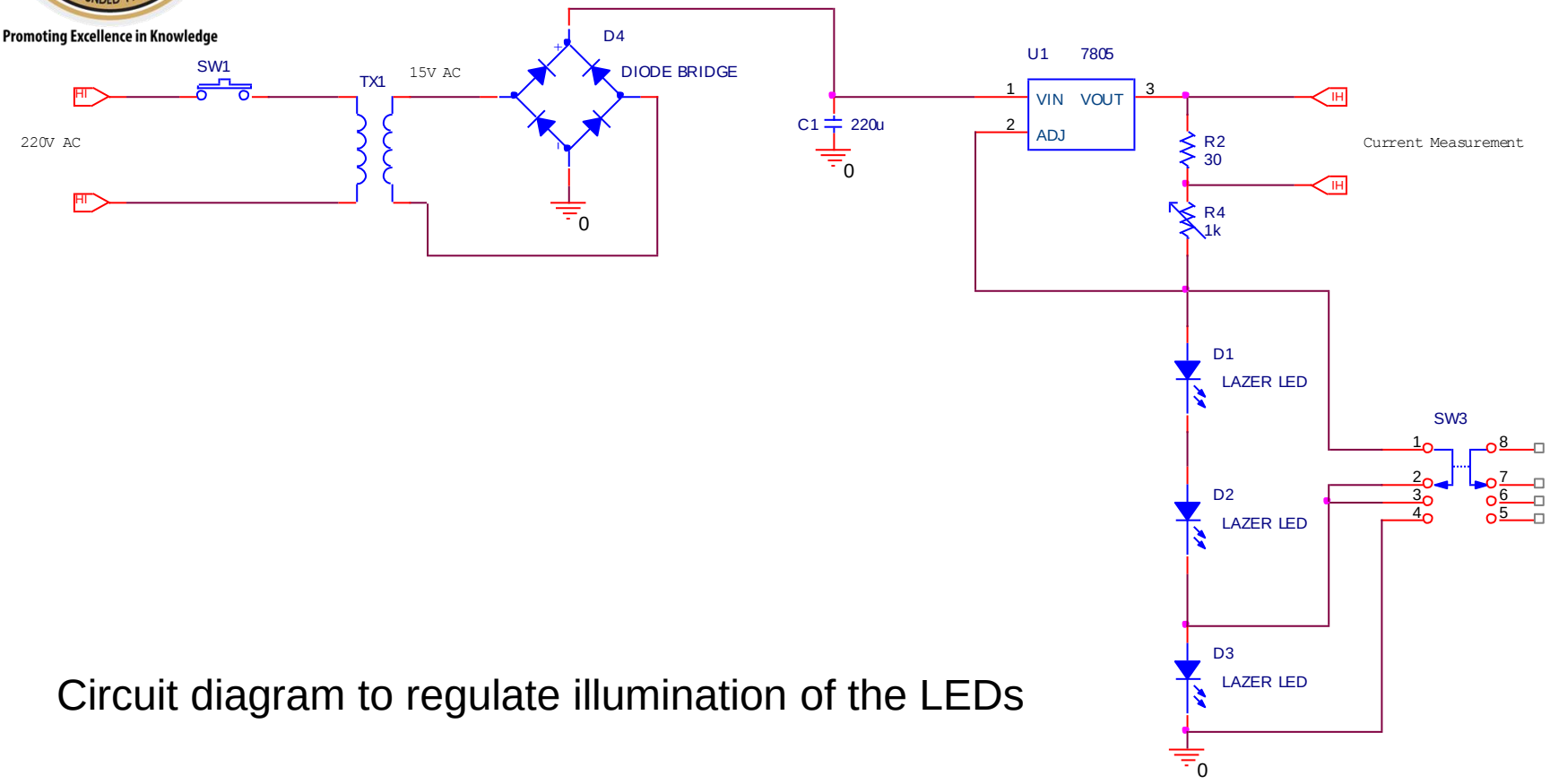
The Developed Chlorophyll Fluorescence Imaging Device With UltraViolet LED

Main Features:

1. Matlab and Labview software for image acquisition and processing.
2. LED at 360nm and 100mW power.
3. Image capture: 1 frame in 40ms. Images recorded were synchronized with delay signal and fluorescence signal to augment sensitivity.
4. Measured Parameters: Fluorescence emission bands or spectra.
5. Detection : CCD Camera or Ocean Optics spectrometer (USB4000)
6. Power Supply: Battery or mains.

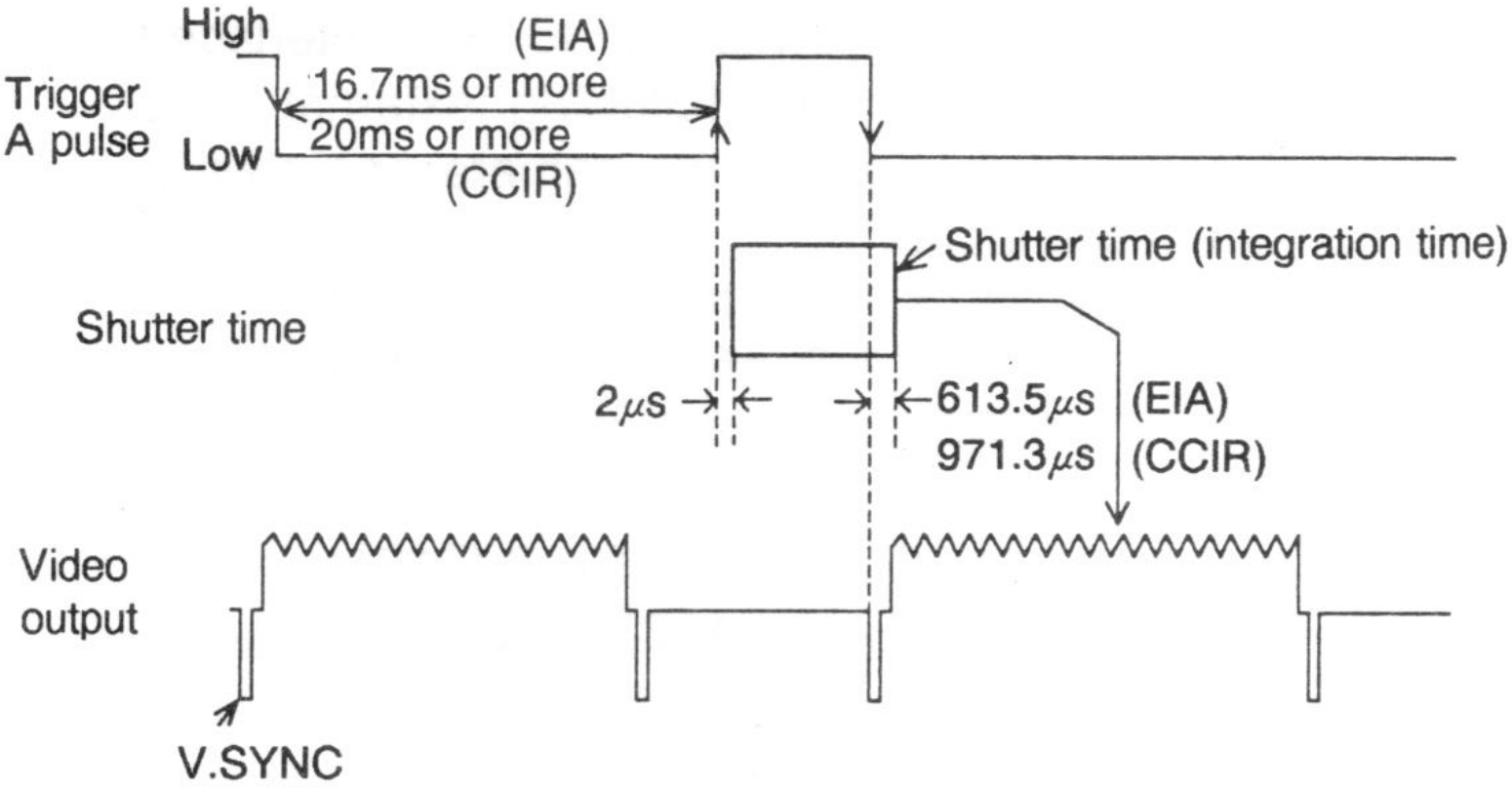
FLUORESCENCE IMAGING WITH UV LED





Circuit diagram to regulate illumination of the LEDs

Camera Triggering



National instruments PCI IMAQ 1405. Resolution 640x480, 8bit

The shutter is started by the rising edge of the the trigger A pulse, and V. SYNC is reset by the falling edge of the trigger A pulse. (After reset, the first field is delivered)

A shutter time is controlled by the duration when the trigger pulse is high.

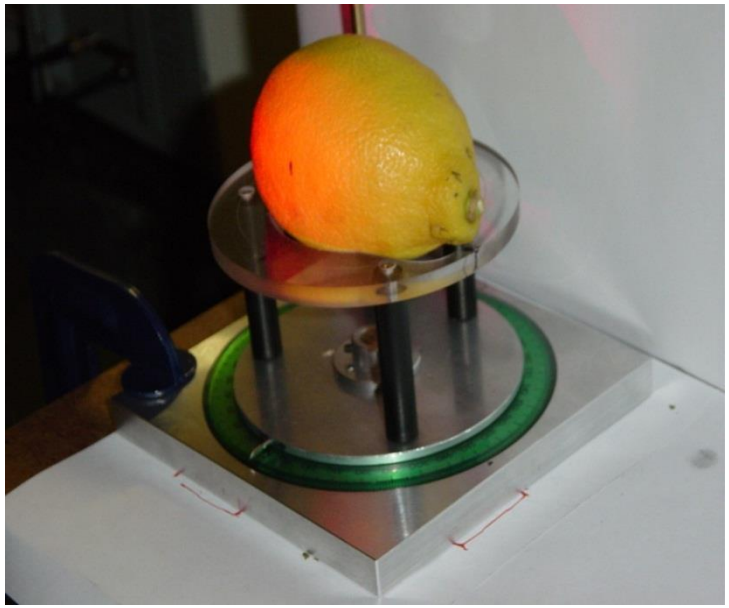
One frame in 40ms synchronized with delay signal and fluorescence signal to augment sensitivity.

Lemon F fresh from super market

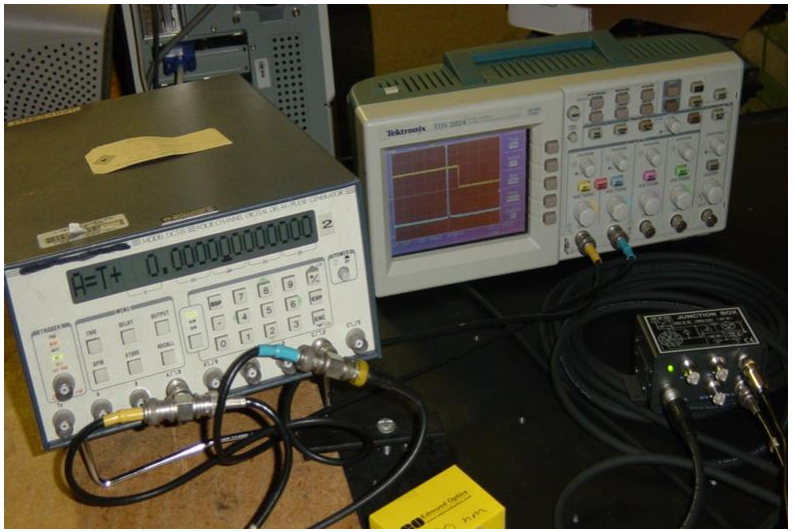
Lemon C taken from super market after 10 days on shelf.

The Rotatable Table

G-Clamped on a Lab Jack

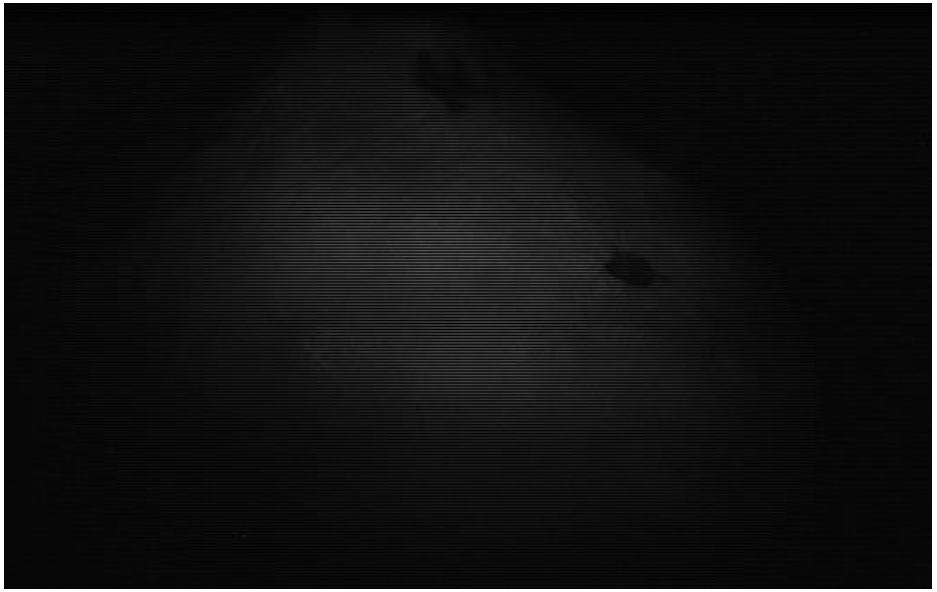


The Delay Pulse Generator Showing Triggering Pulse on the CRO

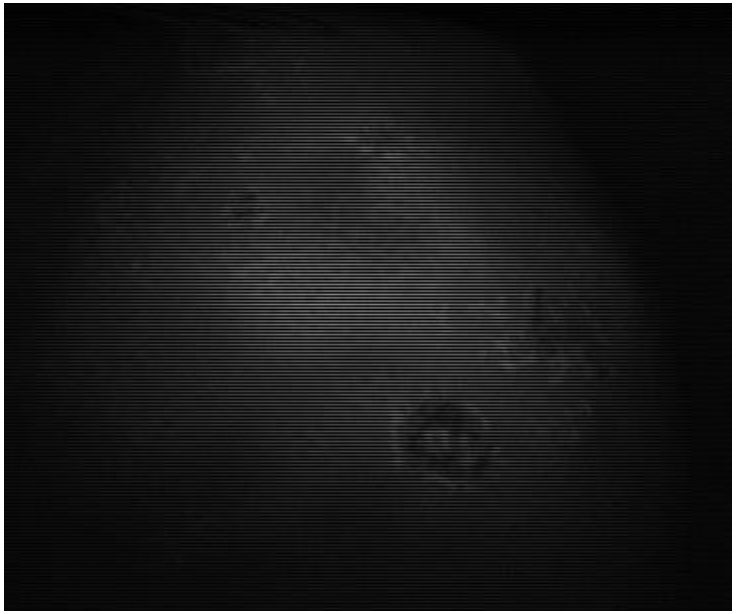


The blue spike on the CRO is the trigger to the PC card from the Delay Generator. The yellow pulse of 120.2 μs width is the camera trigger. The pulse width is 20μs.

Fluorescence imaging of lemon (first day)

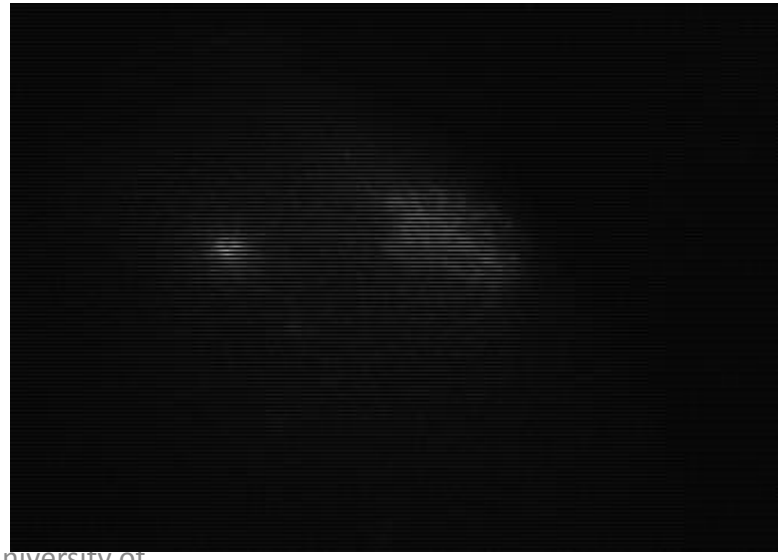


A1



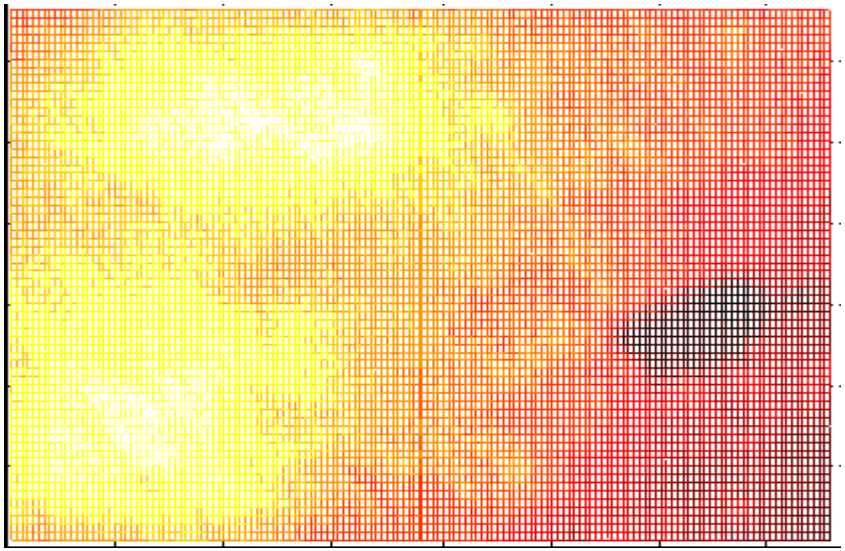
B1

C1

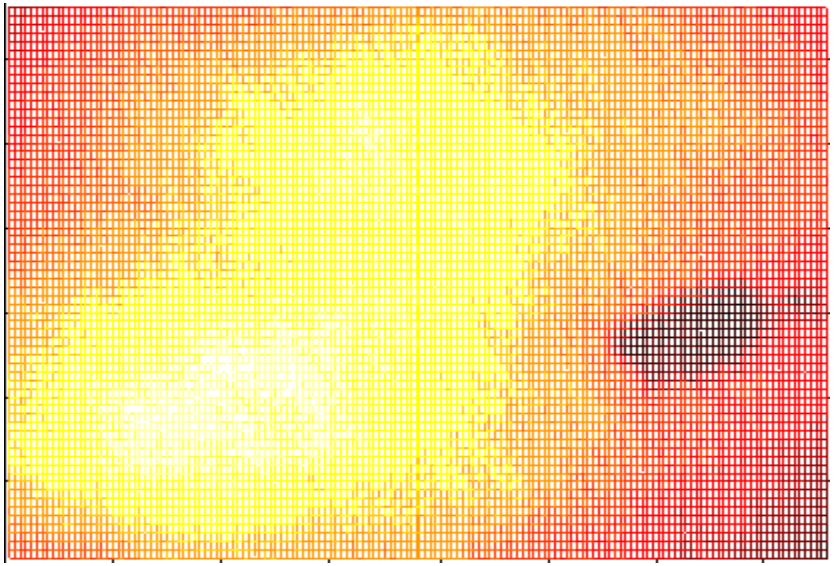


D1

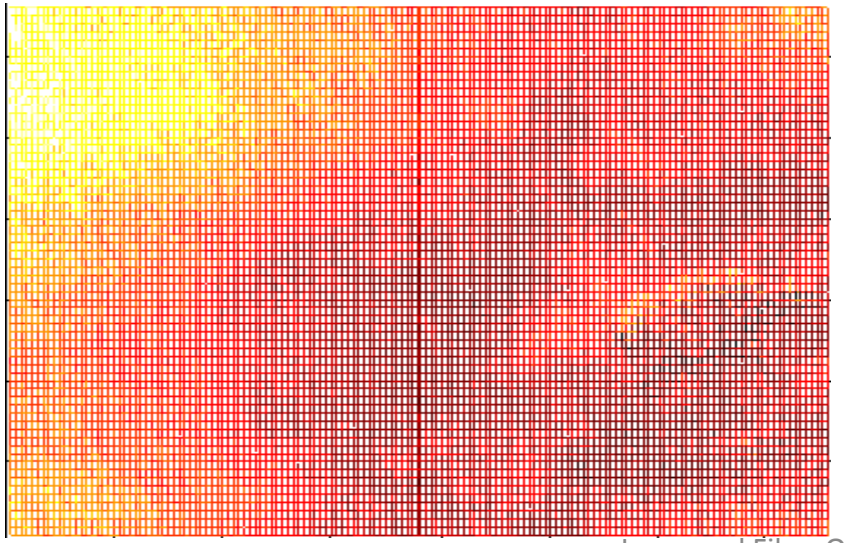
Processed for chlorophyll fluorescence image of lemon at the third day.
Black point is the defect



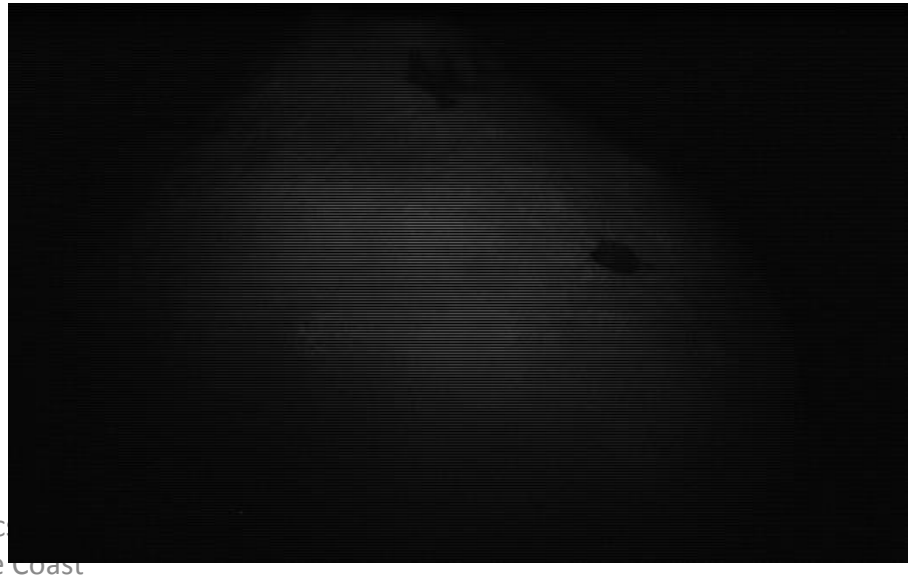
690nm filter LA1

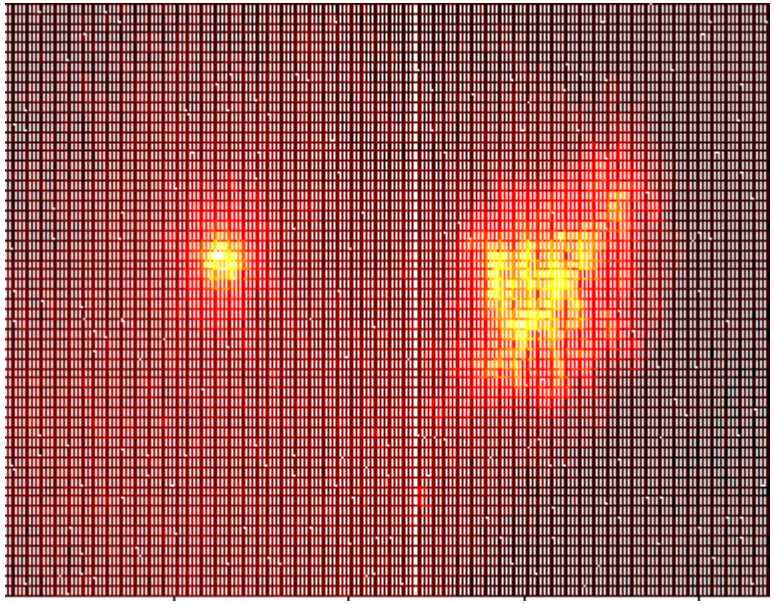


730nm filter LA1

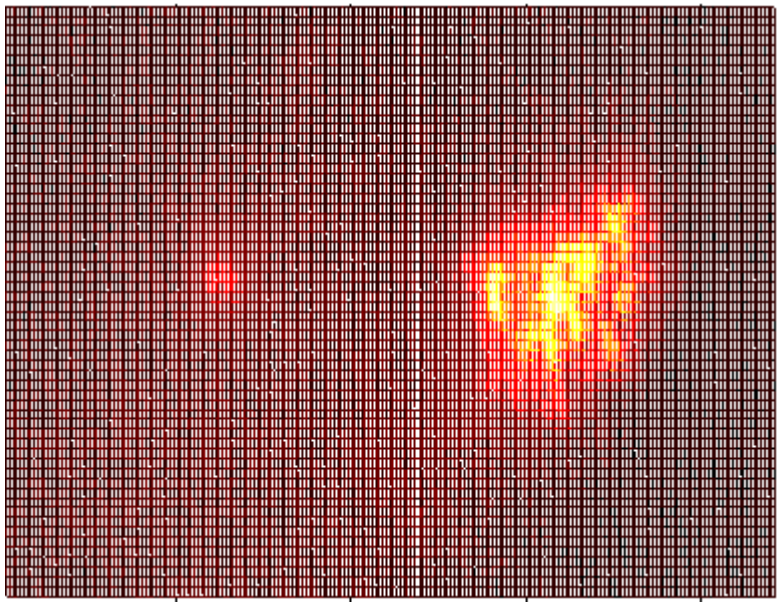


690/730 filter LA1

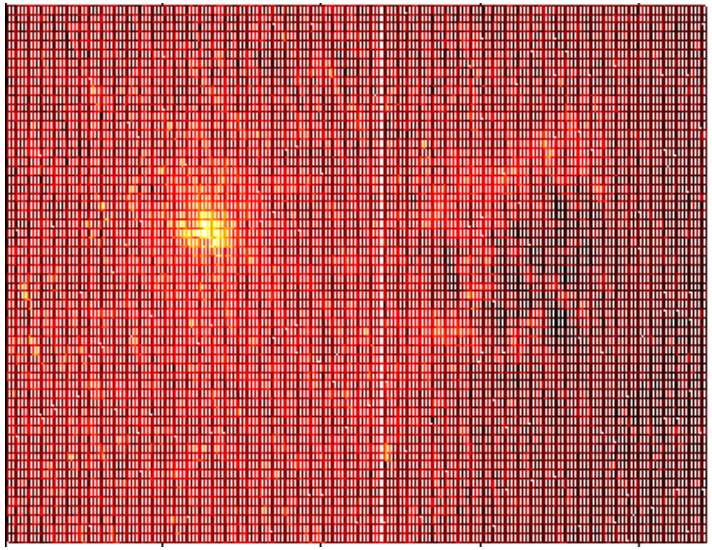




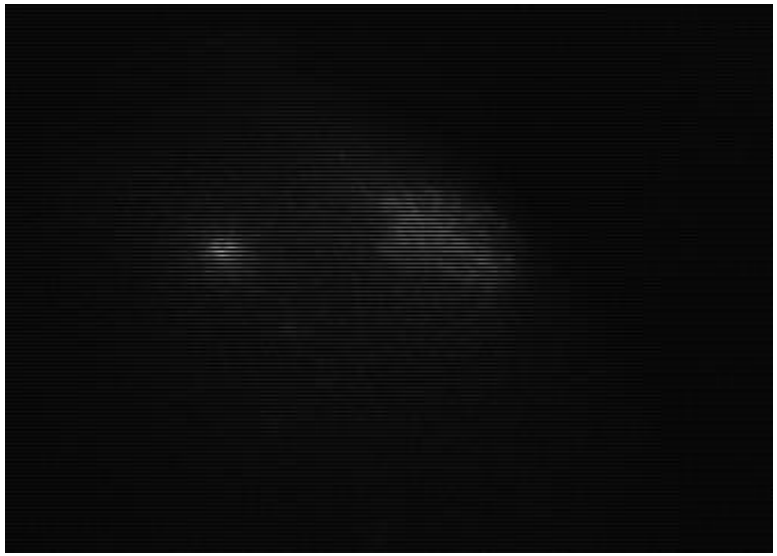
690nm filter LA1



730nm filter LA1



690/730 ratio of LA1



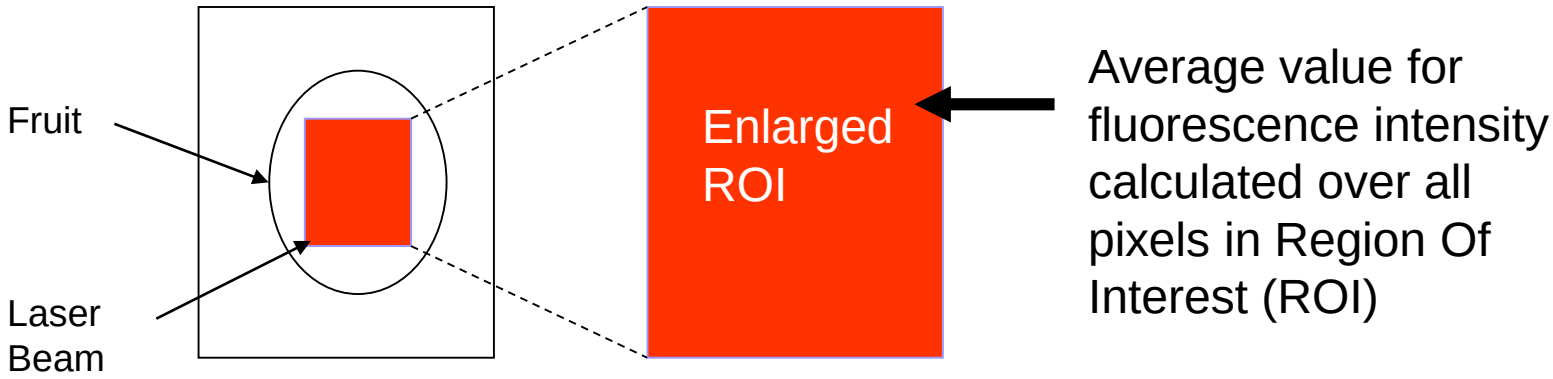
Recorded image

Algorithm

- Using a MatLab Programme, the number of input nodes were five and each was connected to the spectra normalized to corrected pixel values.

  Day (No of Days in dates), Fruit Number (Samples A-F or 1-10), Fruit position (sides 1-6), Filter(690nm or 730nm or 450nm or 550nm), Field (Light or Dark).
- MatLab code using Matrix techniques was used for the calculations.
- All the pixels within a selected region were integrated to give an average value for a given time.

These average values were then used to plot characteristic fluorescence intensity as a function of time for each side of the fruit..



Fluorescence Filtered Image

Data Handling

- **Image Capture** - Sample digitized image was chosen containing intensity levels of varying grey levels ranging from 0 to 255.
- **Image Segmentation** - Region of interest vary with fruit size and the processing box of interest ranges from m×n pixel boxes (e.g. 70×105 to 100 × 220) from the main frame of N×N.
- **Image Analysis**

  If we call x(y) the horizontal pixel coordinates then the ith box will span the coordinates from:

  x_i to x_{i+m} and y_i to y_{i+n}

  Calling I_{xy} the signal observed at pixel (x,y) the total signal is given by:

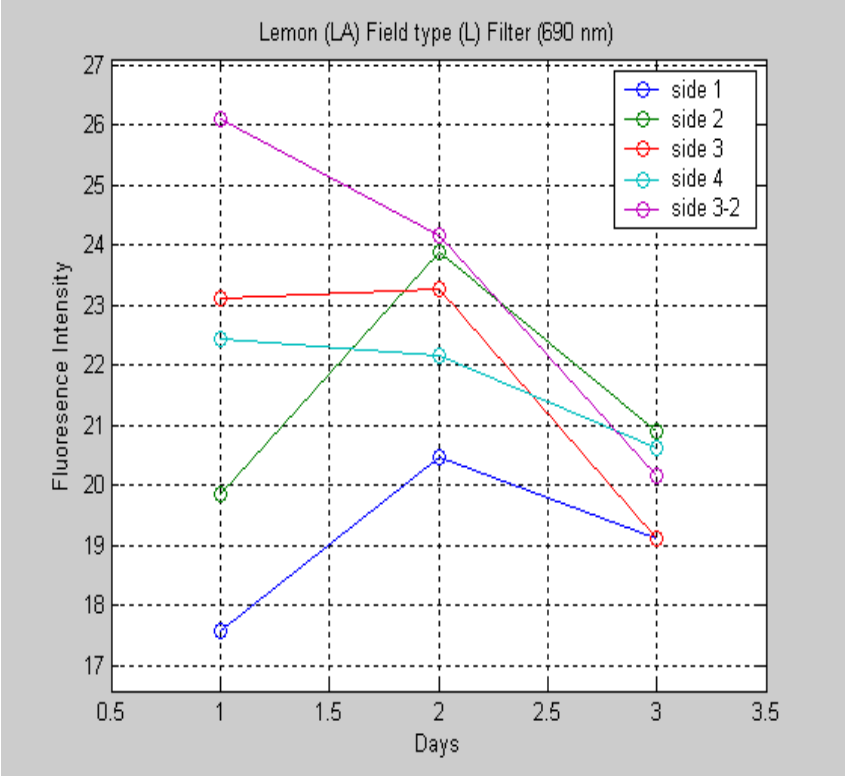
$$I_t = \sum_{x,y=1}^{N} I_{xy}$$

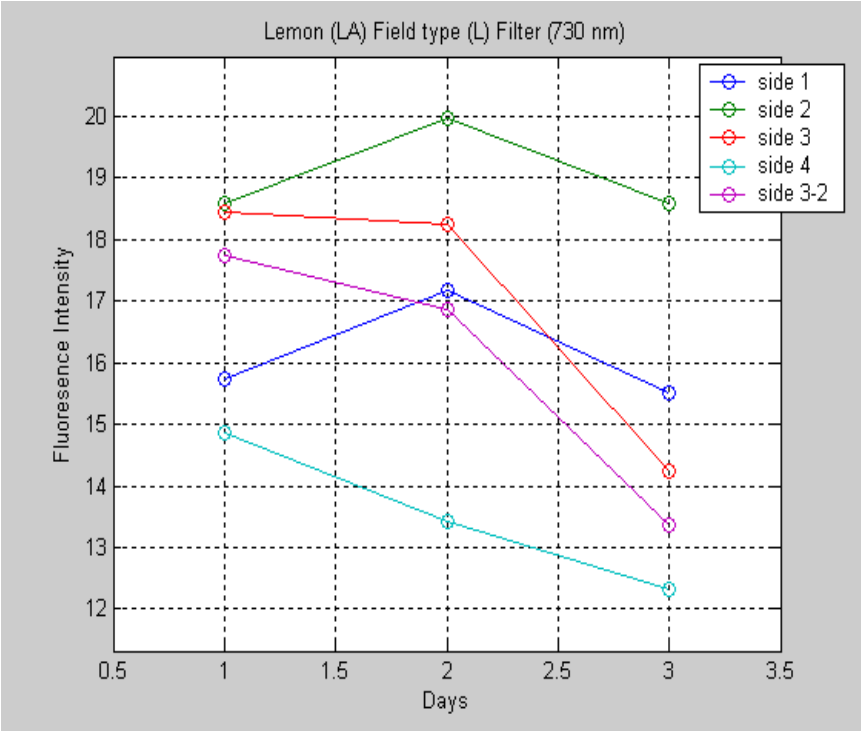
  whereas the local signal observed in each image of the ith box is given by:

$$I_i = \sum_{x=x_i}^{N} \sum_{y=y_i}^{M} I_{xy}$$

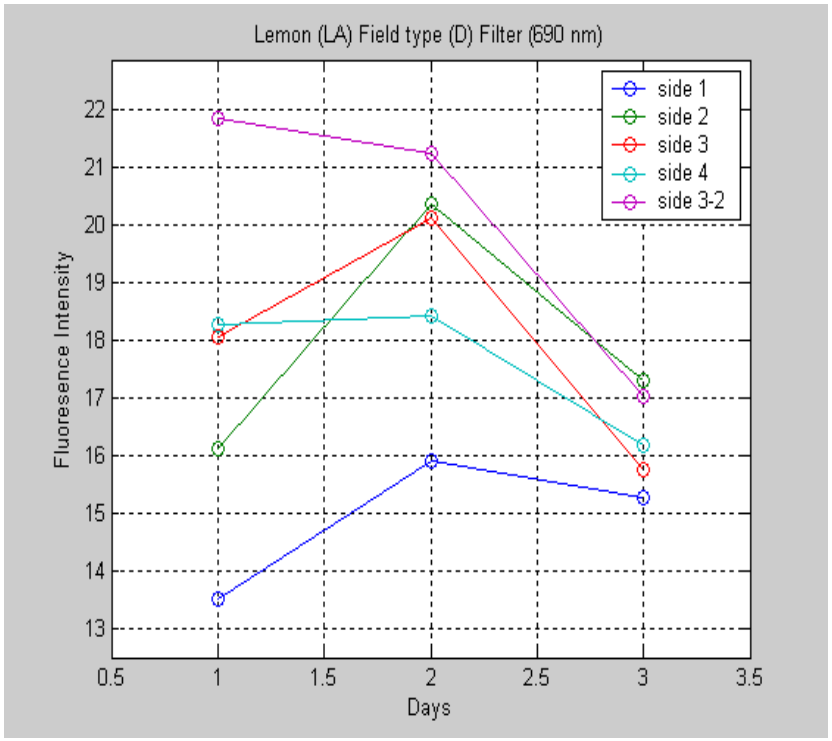
The average Image is given by I_i/I_t .

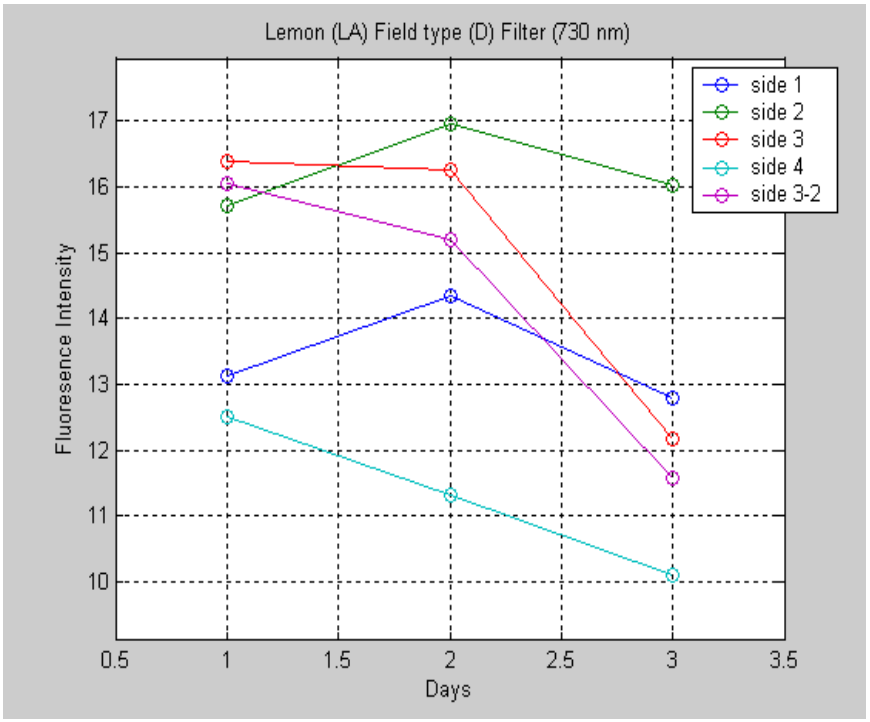
Chlorophyll Fluorescence variation on different points of a lemon at fluorescence filter 690 and 730nm in **background light at different days of ripening**. Side 1,2;3,4,and 3-2 are head, tail and side of the lemon respectively.



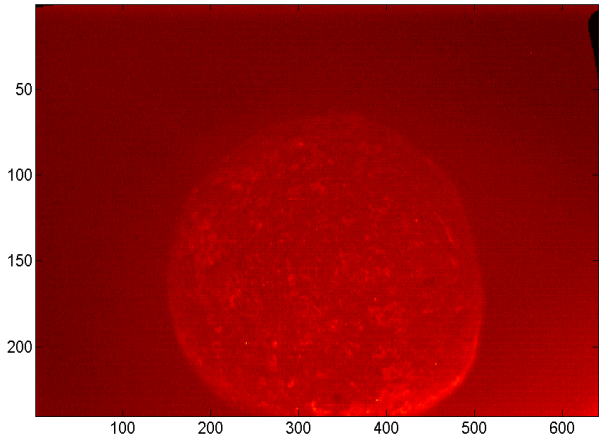


Chlorophyll Fluorescence variation on different points of a lemon at fluorescence filter 690 and 730nm **without background light at different days of ripening**. Sides 1, 2 ;3,4; and 3-2 are head,tail and side of the lemon respectively

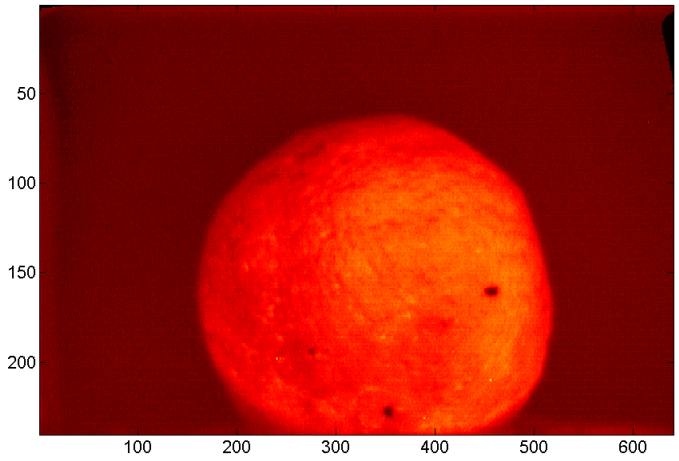




LEMON AT DIFFERENT FILTERS AT ROOM TEMP



450 FILTER



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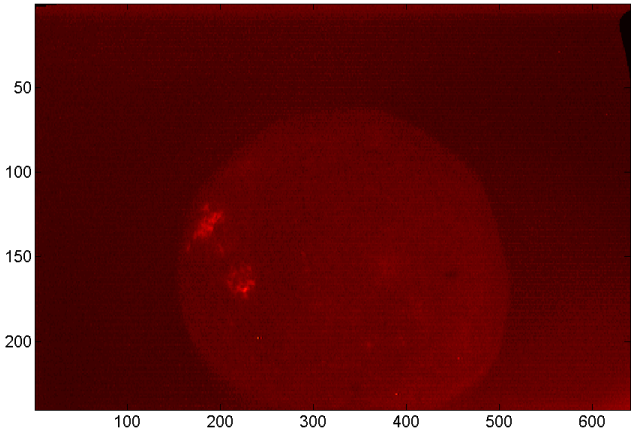
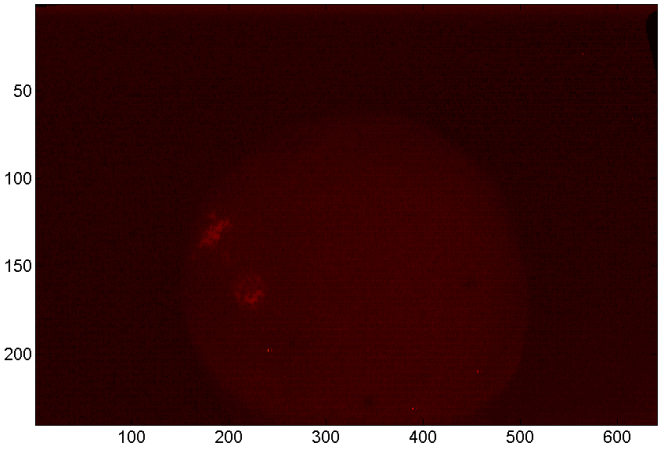
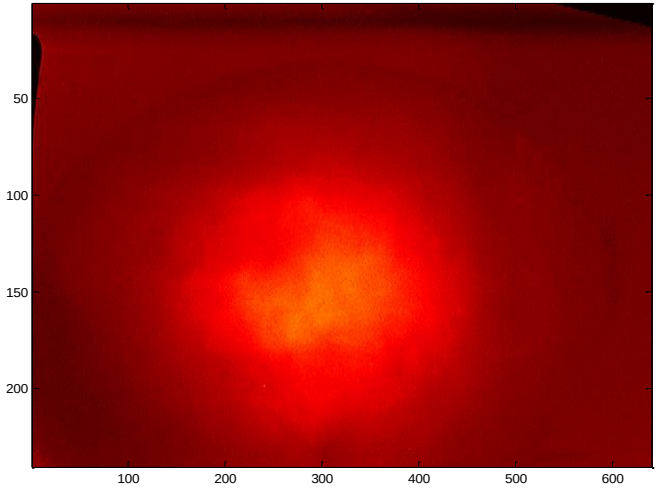


690 FILTER

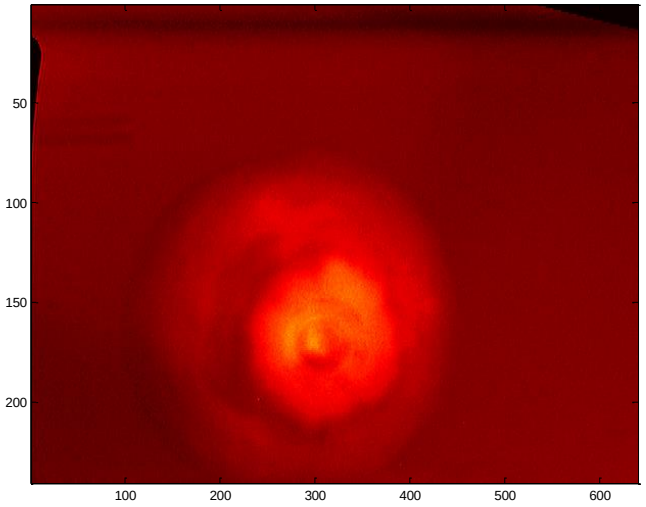


730 FILTER

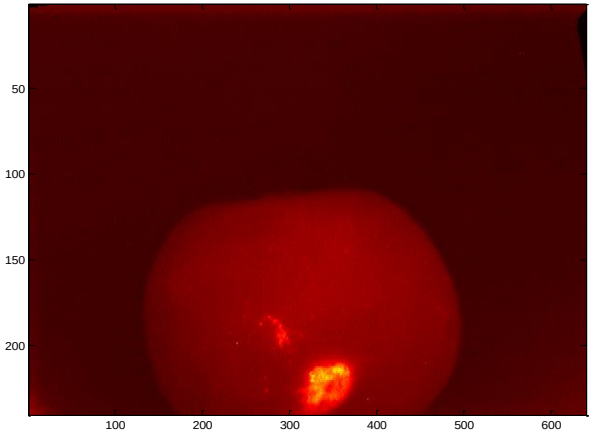
Pixel values of the fluorescence images taken with 690 nm filter



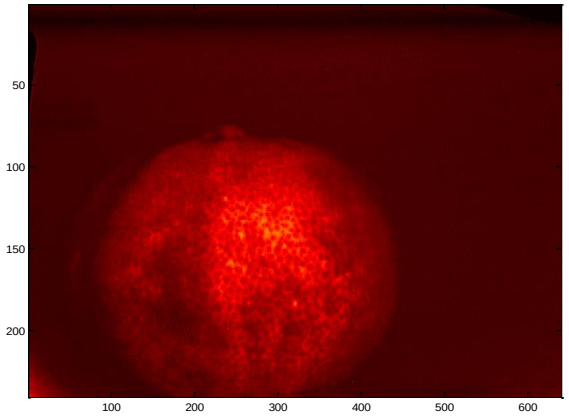
Lemon (LemFD1690L) at 22 deg Celsius



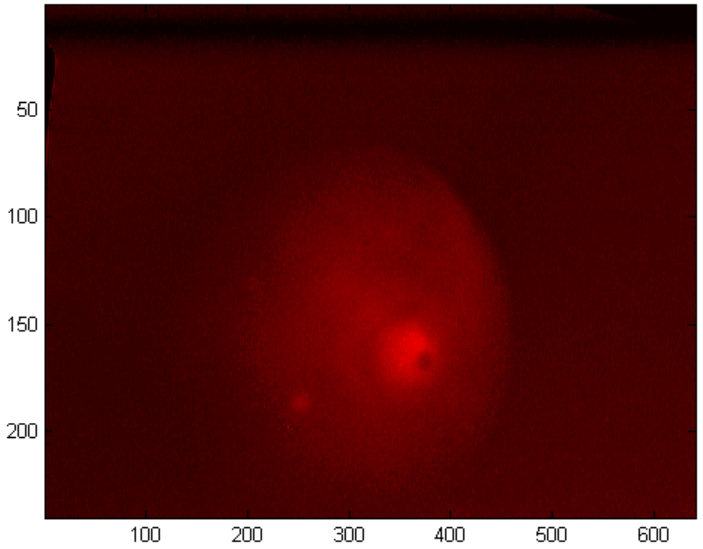
Lemon (LemCB2690L) at 22 deg Celsius

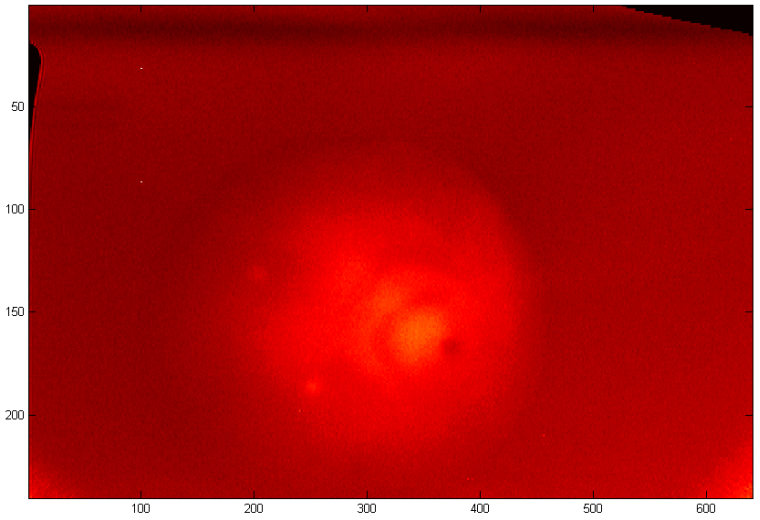


Mandarin (Mahci1690L) with Defect at 6 deg Celsius



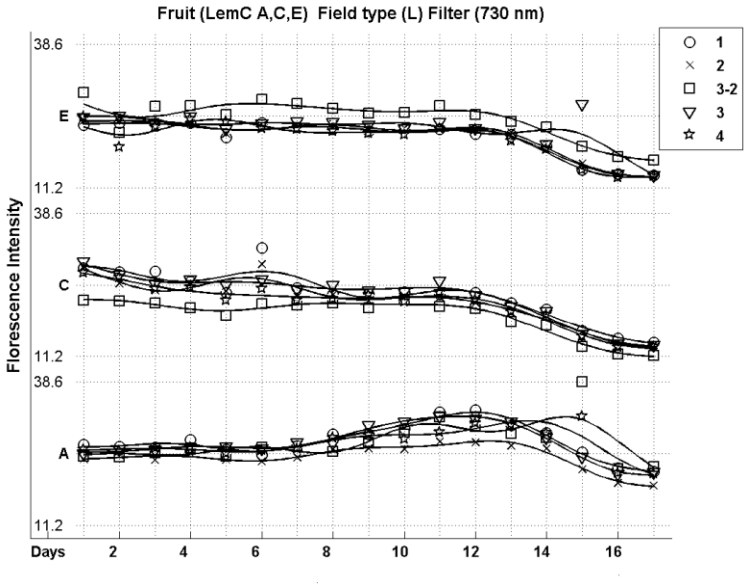
Mandarin (ManBB1690L) at 22 deg Celsius

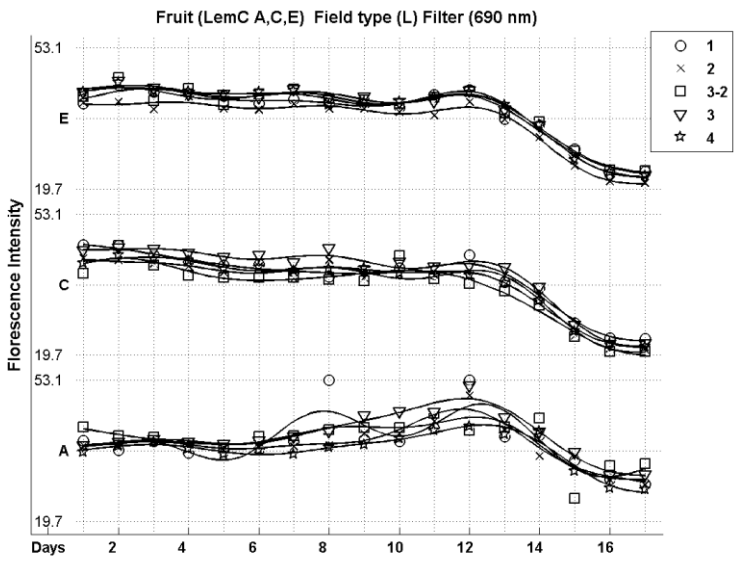


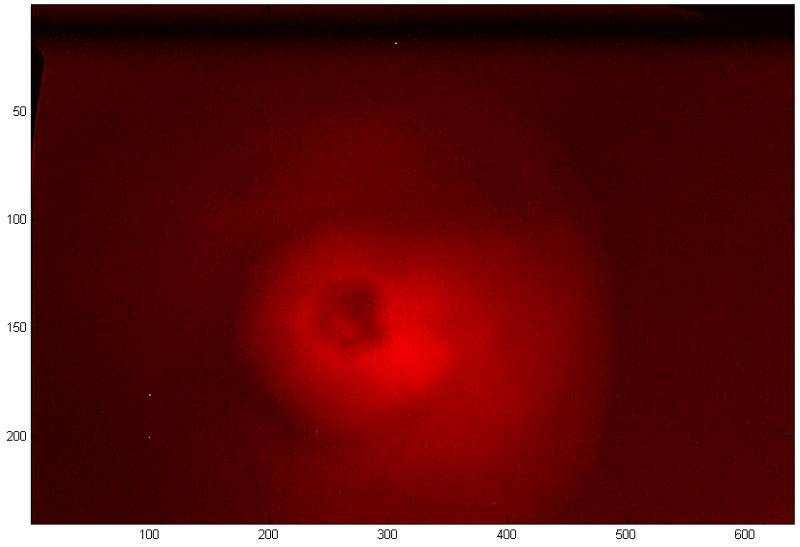


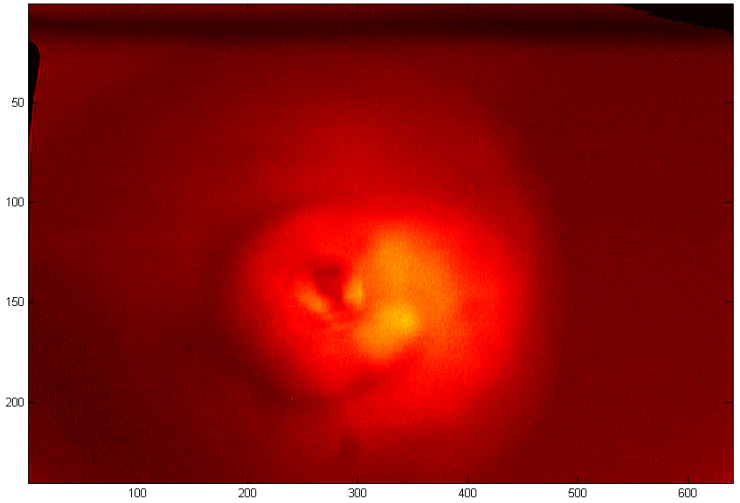
LEMCD4730L

LEMCD4690L

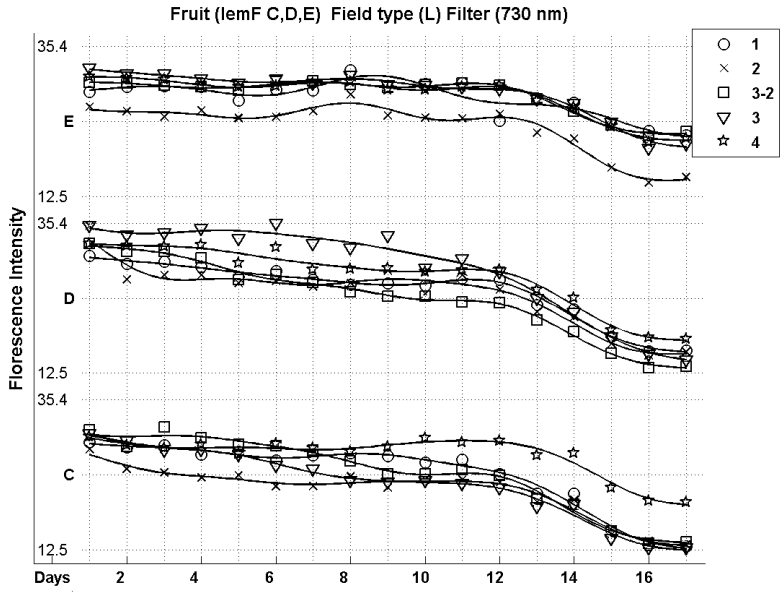




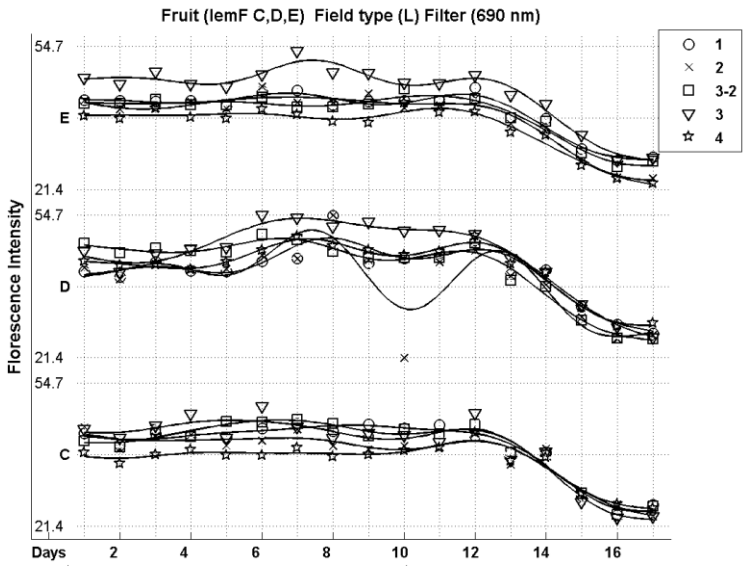


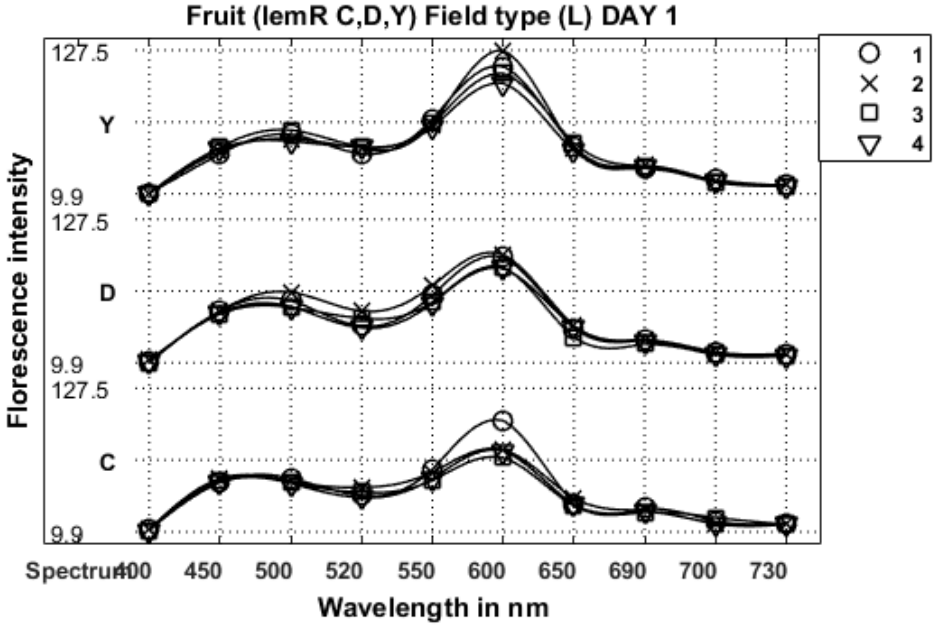


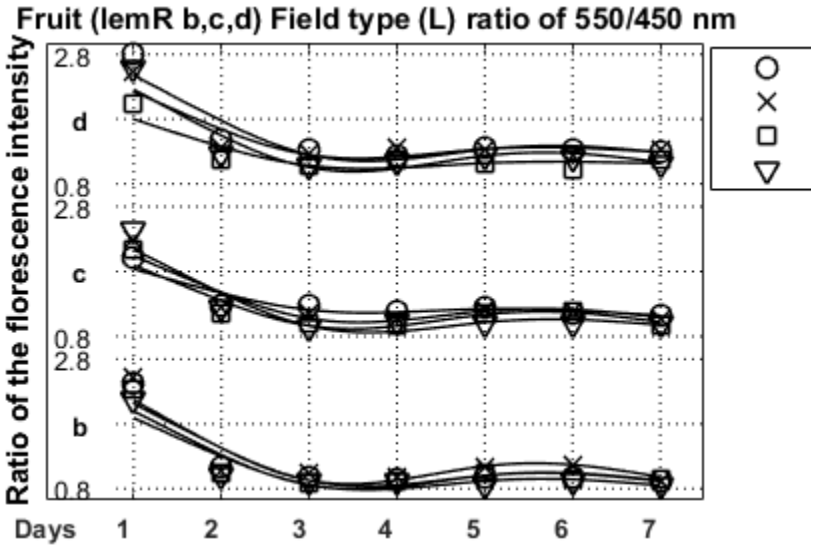
Lemon FE (Filter 730 nm)

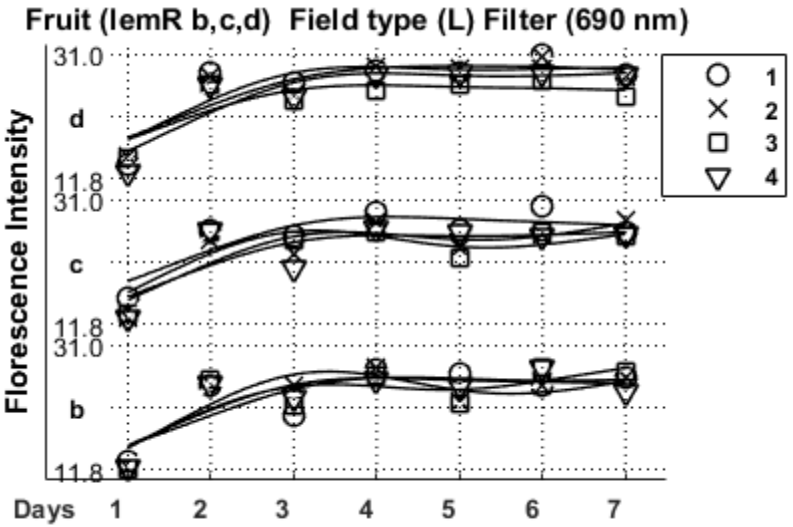


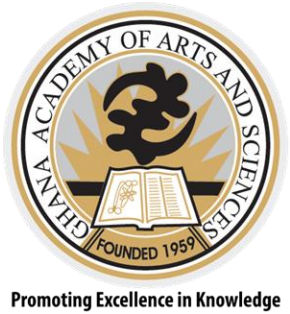
Lemon FE (Filter 690 nm)




Fruit (lemR C,D,Y) Field type (L) DAY 1
Florescence intensity
127.5
Y
9.9
127.5
D
9.9
127.5
C
9.9
1
2
3
4
Spectrum
400
450
500
520
550
600
650
690
700
730
Wavelength in nm


Fruit (lemR b,c,d) Field type (L) ratio of 550/450 nm
Ratio of the florescence intensity
2.8
d
0.8
2.8
c
0.8
2.8
b
0.8
Days
1
2
3
4
5
6
7


Fruit (lemR b,c,d) Field type (L) Filter (690 nm)
Florescence Intensity
31.0
d
11.8
31.0
c
11.8
31.0
b
11.8
1
2
3
4
Days
1
2
3
4
5
6
7



GENERAL APPLICATIONS OF PLANTS AND FRUITS USING CHLOROPHILL IMAGING

Photosynthesis Research **66**: 3–12, 2000.

Regular paper

Kinetic imaging of chlorophyll fluorescence using modulated light

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Received 30 November 1999; accepted in revised form 14 June 2000

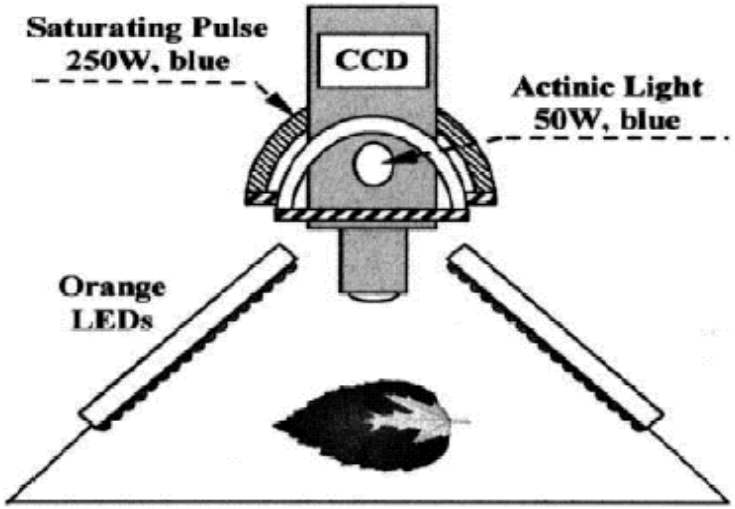

Figure 1. Schematic drawing of the fluorescence imaging instrument. In this configuration, the instrument can image an area 8×10 cm. Measuring flashes (10 μs duration) are generated by two sets of 350 orange light emitting diodes (LED panels). Steady state blue actinic light is generated by a 50 W tungsten-halogen lamp. The duration and intensity of the steady state illumination is user controlled. Saturating pulses of light for determining of F_M and F_M' are generated by a 250 W tungsten-halogen lamp. The 50 W and 250 W lamps are on the opposite side of the camera. Further details concerning the instrument are given in 'Materials and methods'.

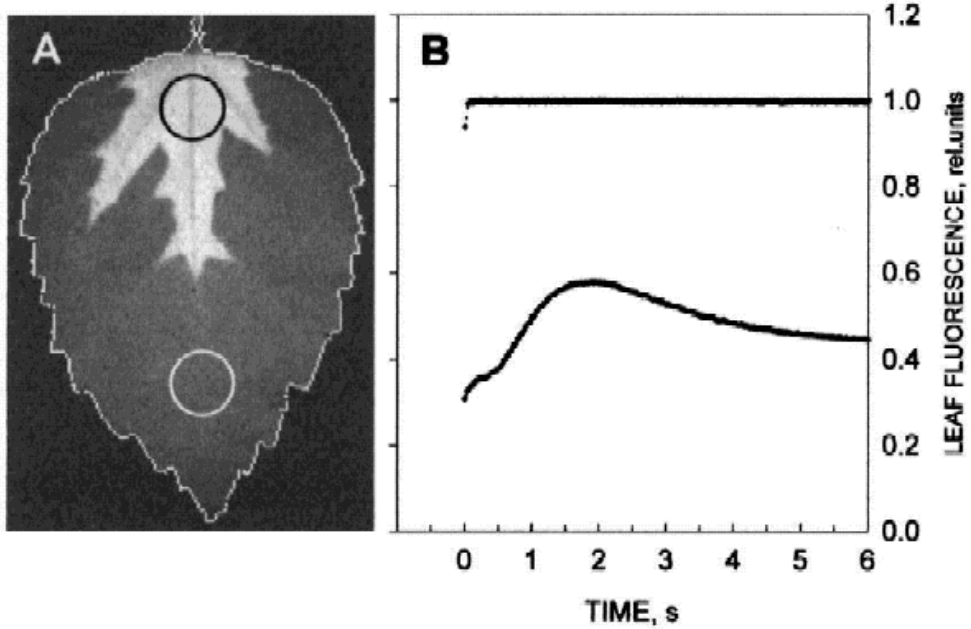

Figure 2. (A) Chlorophyll fluorescence image of *Hibiscus* leaf that had taken up DCMU by having its petiole in water containing the inhibitor (50 μM) overnight. Fluorescence was recorded by the imaging instrument operating in the continuous light mode. The fluorescence transient was induced by illuminating the leaf for 6 s using the orange LED panels (300 μmol photons m^{-2} s^{-1}). During the light exposure, the camera recorded images at a frequency of 1 image/40 ms. The upper trace in (B) shows the fluorescence emission *versus* time for the area indicated by the black circle and corresponds to a region in which DCMU has penetrated. The transient shown is the average of the data within the circle. The bottom trace in (B) is the fluorescence emission *versus* time for the area indicated by the white circle and corresponds to a region that is not inhibited.

Automated detection of fecal contamination of apples based on multispectral fluorescence image fusion ☆

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Abstract

Fluorescence techniques have shown great potential for detecting animal feces on foods. A recently developed field portable multispectral fluorescence imaging system was used to acquire steady-state fluorescence images of feces contaminated apples. Twenty Red Delicious apples encompassing natural color variation were artificially contaminated with dairy cow feces to create five fecal contamination spots on each apple. The feces spots were not clearly visible to the human eye. Multispectral fluorescence images, with wavebands centered at the red emission peaks of cow feces and apples, in addition to blue and green bands, were evaluated to determine an optimal red band for detection of feces contamination spots on apples. The results show that fluorescence emission bands at 670nm provided the greatest potential for the detection of feces contamination on apples. In addition, investigation of multispectral fusion methods indicated that band ratio image of 670nm to 450nm or 550nm improve sensitivity of detection. Two-band ratios along with the use of unsupervised histogram-based thresholding allowed detection of cow feces contaminations on apples regardless of apple colorations with a 100% success rate.

Keywords: Fluorescence imaging; Multispectral; Food safety; Fecal contamination; Apples

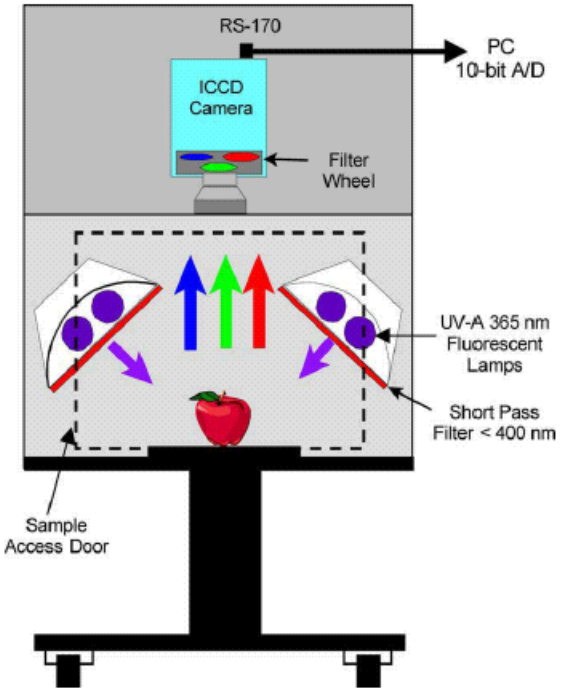

Fig. 1. Schematic diagram of the transportable multispectral steady-state fluorescence imaging system.

Journal of food engineering
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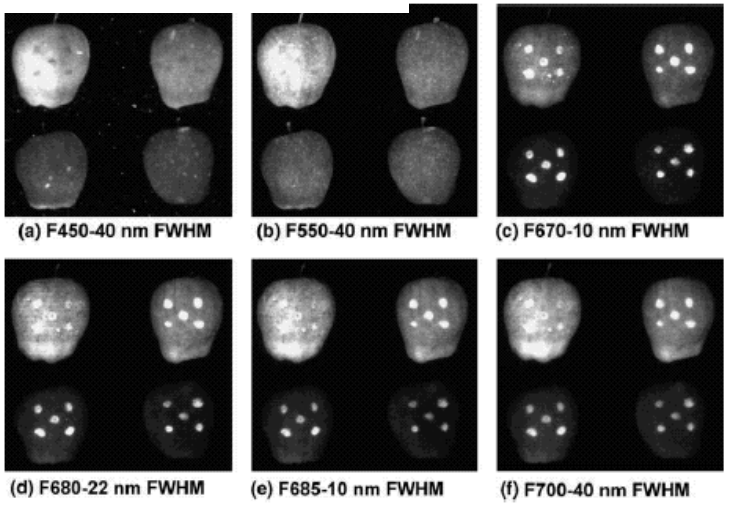

Fig. 2. Steady-state fluorescence images of the shaded (top two apples) and sun-exposed (bottom two) sides of apples with five cow feces contamination spots acquired by the transportable multispectral fluorescence imaging system at F450, F550, F670, F680, F685, and F700. Note that images of individual apples were acquired separately and a composite of four apples, shaded and sun-exposed sides, were created to show the effects of apple color variations in fluorescence responses.

PHOTOSYNTHETICA 38 (4): 571-579, 2000

Postharvest imaging of chlorophyll fluorescence from lemons can be used to predict fruit quality

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Abstract

We demonstrate the feasibility of assaying and predicting post-harvest damage in lemons by monitoring chlorophyll (Chl) fluorescence. Fruit quality was assayed using a commercial instrument that determines photosynthetic performance by imaging Chl fluorescence parameters under different irradiances. Images of Chl fluorescence from individual lemons reveal that photosynthesis is active throughout the post-harvest ripening process. Because photosynthesis is highly sensitive to biotic and abiotic stress, variations in Chl fluorescence parameters over the surface of a lemon fruit can be used to predict areas that will eventually exhibit visible damage. The technique is able to distinguish between mould-infected areas that eventually spread over the surface of the fruit, and damaged areas that do not increase in size during ripening. This study demonstrates the potential for using rapid imaging of Chl fluorescence in post-harvest fruit to develop an automated device that can identify and remove poor quality fruit long before visible damage appears.

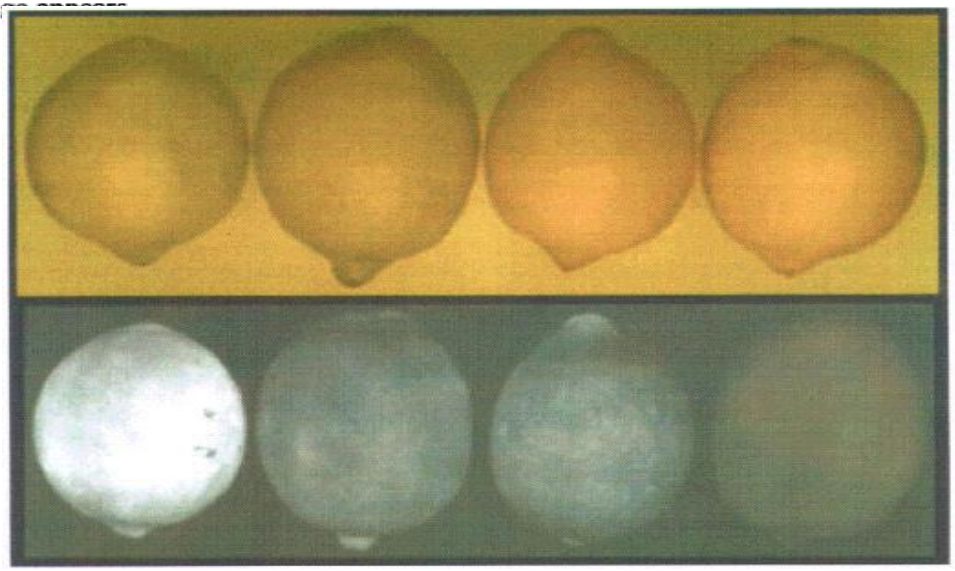

Fig. 1. The *top panel* shows the actual colours (panchromatic) of four lemons at different stages of ripening as the fruit changes from yellow/green to bright yellow. The *bottom panel* shows images of the maximum chlorophyll fluorescence, F_M, of the lemons shown in the top panel. F_M was measured during a 1 s exposure of the fruits at an irradiance of 2000 μmol(photon) m^{-2} s^{-1}.

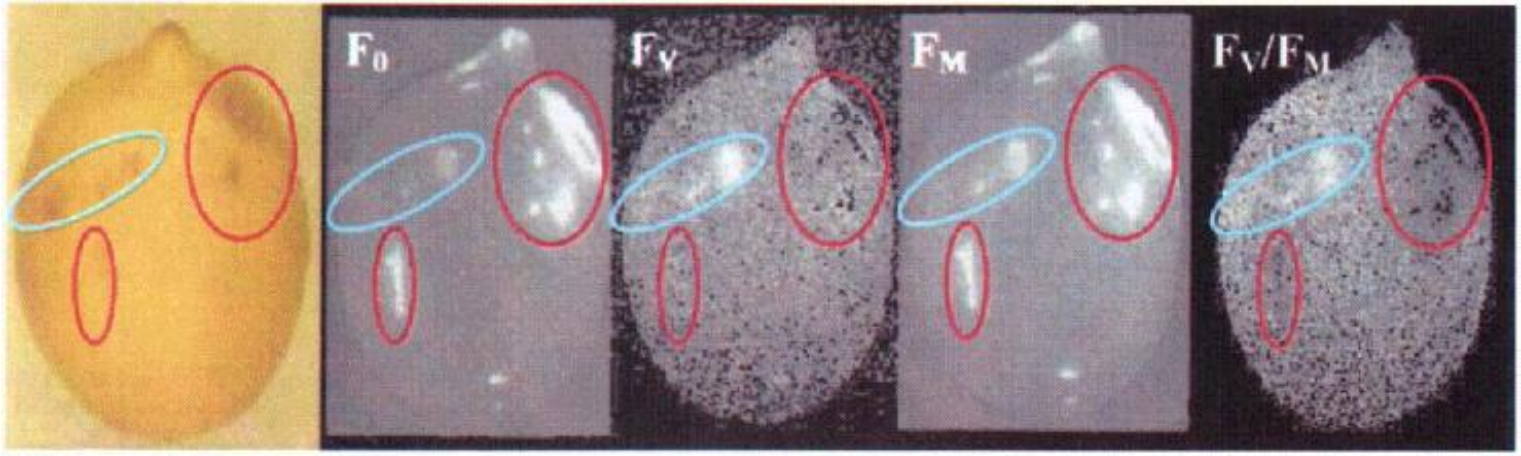

Fig. 3. The photograph shows a lemon exhibiting several areas of visible damage (brown spots). The other panels show the fluorescence images of F_0, F_V, F_M, and F_V/F_M. To increase contrast, the sensitivity (*gray scale*) used to show F_V was 4 times larger than in the F_0 and F_M images. The ratio F_V/F_M is shown using gray scale where black is $F_V/F_M = 0$ and white is 1. *Red-circled areas* developed 2 d later a visible green mould growth. *Blue circled area* remained stable.

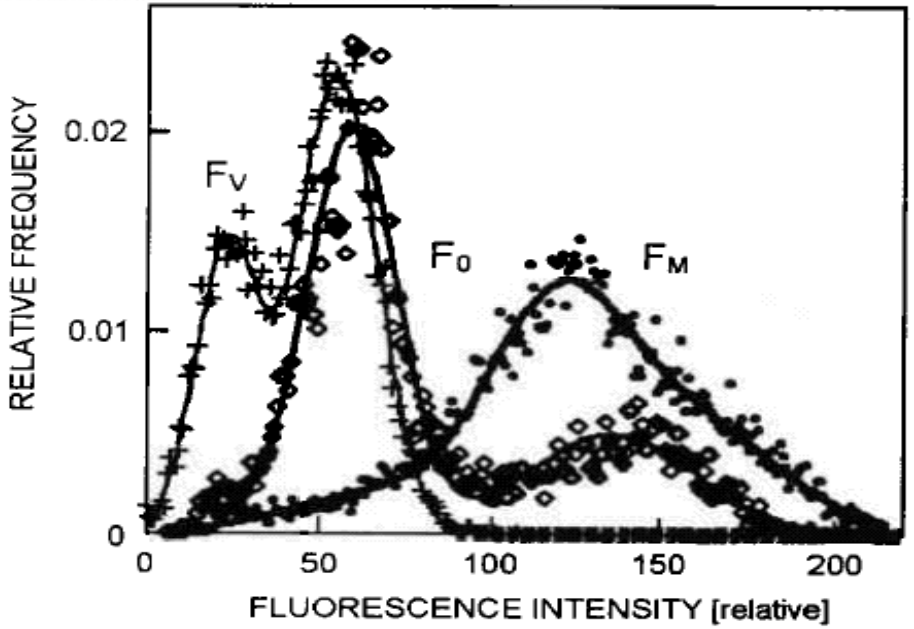

Fig. 5. The frequency (*Y-axis*) of various levels of the fluorescence parameters (*X-axis*) is shown for a lemon infected by the green mould *Penicillium digitatum*. The values correspond to the 3rd d of infection.

PHOTOSYNTHETICA 43 (3): 355-369, 2005

Chlorophyll fluorescence imaging of photosynthetic activity with the flash-lamp fluorescence imaging system

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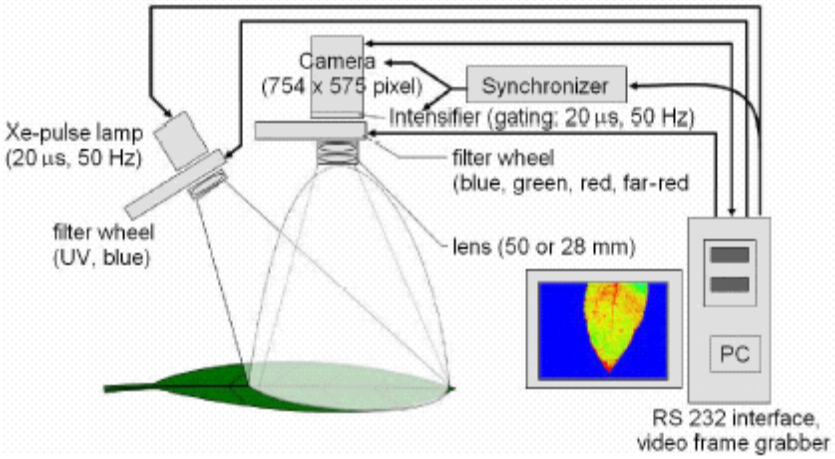

Fig. 2. The flash-lamp fluorescence imaging system (FL-FIS) for acquiring blue (F_{440}), green (F_{520}), red (F_{690}), and far-red (F_{740}) fluorescence images of green leaves. A pulsed xenon lamp with UV-transmission filter is used as an excitation source when images of the four fluorescence bands are collected. A CCD video camera with an intensifier collects, in each fluorescence band, the emitted fluorescence of several hundred thousand pixels per leaf. The images are processed by the image processing system of a PC. If only fluorescence images of the red and far-red Chl fluorescence are needed, a blue filter is applied to the filter wheel in front of the xenon lamp. For the spectral range of both filters see Fig. 3.

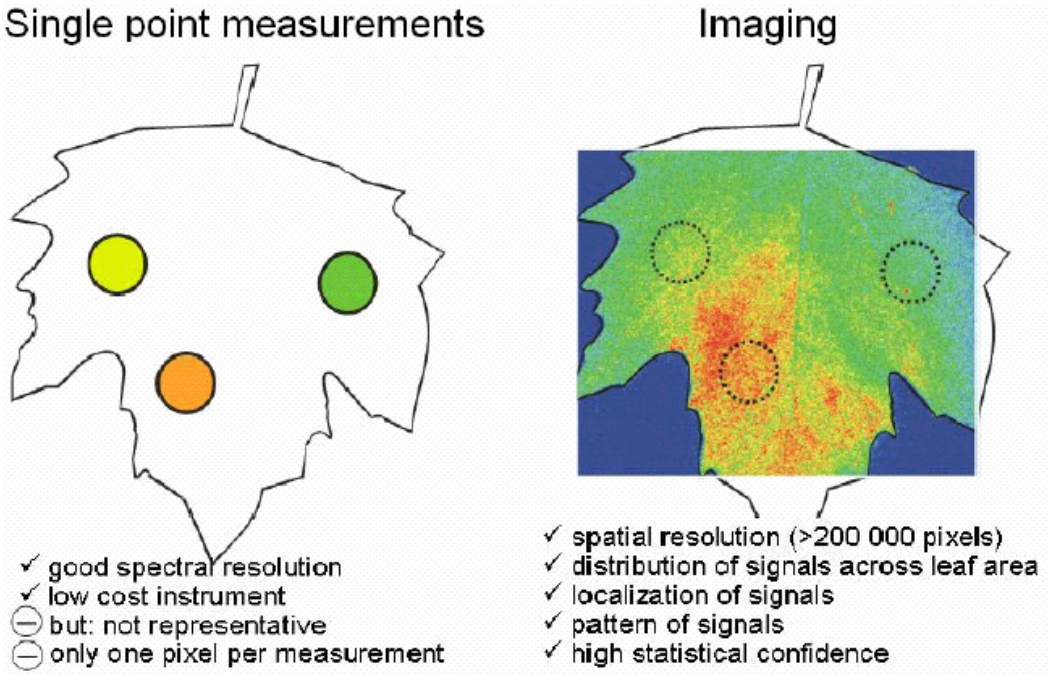

Fig. 13. The advantages and superiority of leaf fluorescence imaging compared to the fluorescence measurement at single leaf spots here shown for a maple leaf. The spatial heterogeneity of the Chl fluorescence signals and ratios shows up *via* imaging, but not at all or not necessarily *via* single leaf point measurements, even if several points of a leaf are measured.

Conclusions and Recommendations

- Fluorescence methods for evaluating maturity in fruits is the lose of chlorophyll as they ripen or mature and are sensitive to chilling injury and stress.
- Chlorophyll Fluorescence can detect surface damage on fruits in vivo or extract chlorophyll from the peel in vitro for analysis in the near infra red region.
- Shelf-life of fruits can be determined and expiry date predicted
- Presence of toxic detected

harvest- ripening and maturity

handling –chemical or quarantine treatment and storage

post-harvest- physiological stages and technological handling

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• The Council, Ghana Academy of Arts and Sciences, Ghana
•AASSA-NAST PHILLIPINES INTERNATIONAL SYMPOSIUM ORGANISERS

THANK YOU