FINAL PROJECT REPORT
WTFRC Project Number: PH-04-0442

Project Title: Hyperspectral Reflectance and Fluorescence for Assessing Apple Maturity

PI:	Renfu Lu	**Co-PI(2):**	Randolph M. Beaudry
Organization:	USDA/ARS	**Organization:**	Michigan State Univ.
Telephone/email:	517-432-8062	**Telephone/email:**	517-355-5191 ex. 303
Address:	224 Farrall Hall	**Address:**	A21 PSSB
Address 2:	Michigan State Univ.	**Address 2:**	
City:	East Lansing	**City:**	East Lansing
State/Province/Zip	MI 48824	**State/Province/Zip:**	MI 48824

Budget History:

Item	**Year 1:** 2004	**Year 2:** 2005	**Year 3:** 2006
Salaries	18,400	18,952	9,760
Benefits	6,624	6,823	3,514
Wages	4,576	4,713	2,428
Benefits			
Equipment			
Supplies	1,500	1,500	750
Travel		800	400
Miscellaneous			
Total	31,100	32,788	16,852

OBJECTIVES

The overall objective of the project is to develop a sensing technique of integrating reflectance (or interactance) and fluorescence for measuring multiple apple quality parameters including skin and flesh color, fruit firmness, soluble solids, starch, and acid. Specific objectives are to:

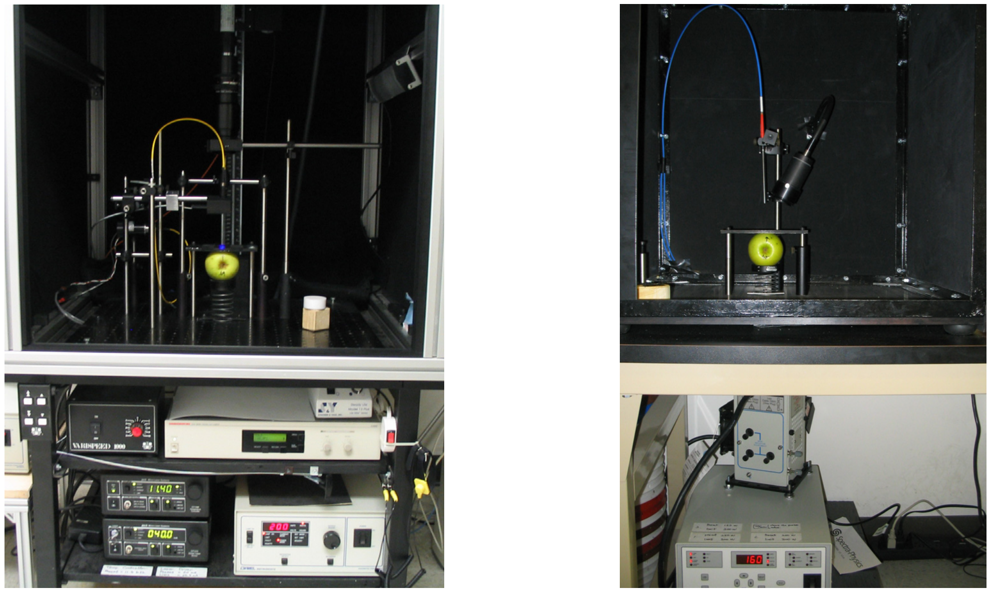
- Investigate the feasibility of using single sensing methods to measure both reflectance (or interactance) and fluorescence from apple fruit;
- Develop mathematical methods and algorithms to integrate reflectance and fluorescence data for predicting fruit maturity parameters;
- Develop a prototype for measuring reflectance and fluorescence to predict fruit maturity parameters including fruit firmness, soluble solids, starch, acid, and skin and flesh color.

SIGNIFICANT FINDINGS

- Reflectance and fluorescence are useful for measuring apple quality parameters including skin and flesh color, fruit firmness, soluble solids, starch, and acid. However, each sensing mode alone may not be sufficient for accurate measurement of all maturity parameters.
- Reflectance (or interactance) is generally better than fluorescence for the measurement of fruit maturity, which is especially evident for firmness, soluble solids and acid measurement.
- Integration of reflectance and fluorescence improves predictions of all maturity parameters; the improvements are more pronounced for firmness, starch, and acid predictions than for soluble solids and skin and flesh color. These improvements are critical since neither reflectance nor fluorescence can provide good predictions of fruit firmness and starch.
- Reflectance or fluorescence alone may be sufficient for providing good predictions of fruit skin and flesh color.
- A compact laboratory prototype was assembled and tested, which is capable to acquire both reflectance (interactance) and fluorescence spectra. The prototype provides excellent measurements of fruit skin and flesh color and soluble solids content. The prototype also gives relatively good prediction of fruit firmness.
- Reflectance and fluorescence techniques are complementary and the integration of the two sensing techniques can provide a better means for measuring multiple quality attributes of apples.

METHODS

In the first year, we assembled and tested a hyperspectral reflectance and laser-induced fluorescence (R/LIF) imaging system (Fig. 1a) and a reflectance and UV-induced fluorescence (R/UV-F) spectroscopic system (Fig. 1b). The R/LIF imaging system consisted of a hyperspectral imaging unit and two separate light sources. A blue laser was used for inducing fluorescence in apples and a broadband light source for acquiring the reflectance scattering images. The R/LIF imaging system was capable of acquiring both reflectance and fluorescence scattering images from apples over the visible and short-wave near-infrared region between 500 and 1000 nm. The R/UV-F spectroscopic



(a) (b)

Figure 1. The hyperspectral reflectance and laser- induced fluorescence (R/LIF) imaging system (a) and the reflectance and UV-induced fluorescence (R/UV-F) spectroscopic system (b).

system also had two sensing modes: reflectance and fluorescence. A xenon lamp was used as an ultra-violet (UV) light source to induce fluorescence and a broadband halogen tungsten light was used for reflectance. A low-cost spectrometer was used in the R/UV-F system for acquiring both reflectance and fluorescence spectra from each fruit.

The two systems were tested on after-storage ‘Golden Delicious’ and ‘Red Delicious’ apples in two separate experiments as a first step in developing an integrated reflectance/fluorescence sensing technique for measuring multiple fruit quality parameters. In the first experiment, the R/UV-F spectroscopic system was used to collect diffuse reflectance and fluorescence spectra from 300 ‘Golden Delicious’ and 350 ‘Red Delicious’ apples, which had been kept in controlled atmosphere storage for 4-5 months. In the second experiment, the R/LIF imaging system was used for collecting hyperspectral reflectance and fluorescence scattering images from 400 ‘Golden Delicious’ apples. After reflectance/fluorescence images and spectra had been collected from individual apples, standard destructive methods were used for measuring multiple quality parameters of each fruit, including skin and flesh color, fruit firmness, soluble solids (SS), and titratable acid. Starch pattern index was not measured because these apples had been in CA storage for 4-5 months and were not suitable for starch determination.

After all data had been collected, prediction models for individual quality parameters were first developed from either reflectance or fluorescence data. Prediction models that combine reflectance and fluorescence data were then developed. Single sensing models and the combined models were compared to determine how they measured fruit quality parameters.

Based on the experimental findings from after-storage apples in the first year study, improvements to the R/LIF imaging system and the R/UV-F spectroscopic system were made before they were used to test freshly harvested apples in the 2005 harvest season. These improvements allowed better control of UV or laser illuminations for fluorescence measurements and faster acquisition of reflectance and fluorescence data. Furthermore, a calibration procedure was developed to compensate for the effect of light source fluctuation on fluorescence measurements. For the R/UV-F system, the sensing mode changed from reflectance (light illumination and detection is on the same area of the sample) to interactance or semi-transmittance (the light detection area is separate from the illumination area). The use of the interactance mode allowed better light penetration into the fruit and thus improved the measurement of flesh properties. The two improved systems were tested on 750 'Golden Delicious' apples within 24 hours after harvest. Apples were harvested once a week and the first harvest started two weeks before the optimal commercial harvest time. Five harvests were made over a period of four weeks in the fall of 2005.

Prediction models using both the artificial neural networks and statistical methods were developed for individual quality parameters using either reflectance (or interactance) or fluorescence data. Prediction models were also developed using the combined reflectance and fluorescence data. A method that is different from the one used in the previous study was used for analyzing the combined data of reflectance and fluorescence. We first applied a mathematical method (called principal component analysis or PCA) to extract essential information (or principal components or PCs) for each set of data. The two sets of PCs from reflectance and fluorescence were input into an artificial neural network. Once the neural network was properly trained, it was then used to predict maturity parameters of other samples. Finally, single sensing models and the combined models were compared to determine their performance in predicting fruit quality parameters.

With the promising results from the first one and half year study of after-storage and freshly harvested apples, our focus in last year was to assemble a laboratory interactance and UV-induced fluorescence prototype. The prototype consisted of two miniature visible/NIR spectrometers and two light sources (one for interactance measurement and the other for fluorescence measurement) (Fig. 2). The use of two spectrometers allows more efficient measurement of interactance and fluorescence since fluorescence signals are often much weaker than the interactance signals. The prototype was able to acquire both interactance and fluorescence within a short time (<250 ms). The spectrometers used were compact (about the size of a wallet) and covered the spectral range between 400 nm and 1100 nm.

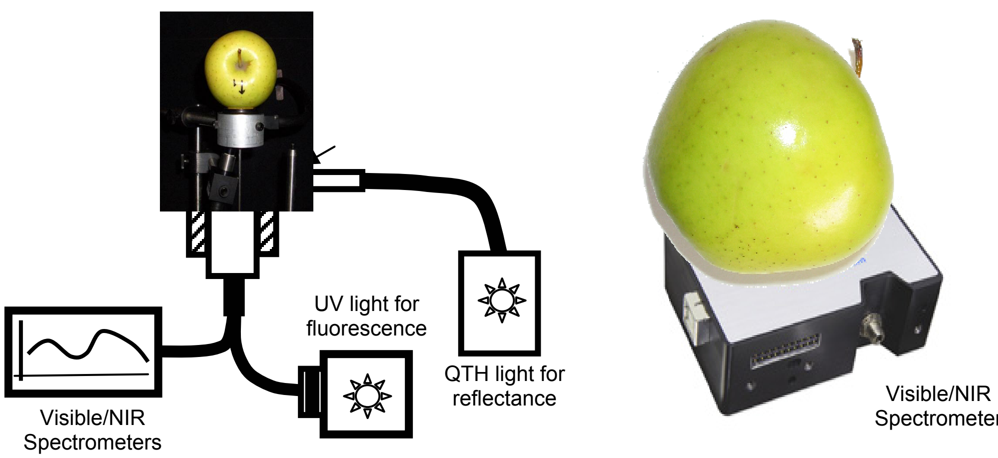


Figure 2. Schematic of a laboratory prototype for measuring interactance and fluorescence spectra from apple.

This prototype was used to collect interactance and fluorescence data from 'Golden Delicious' apples in the 2006 harvest season. Nine hundred 'Golden Delicious' apples were harvested over a period of four weeks. Interactance and fluorescence measurements were performed within 24 hours after harvest. After spectral measurements, standard destructive tests were performed on each fruit to measure individual maturity parameters (skin and flesh color, fruit firmness and soluble solids). The 2006 season was quite unusual; the starch conversion phenomenon was not observed in Golden Delicious apples during the four-week harvest period. As a result, starch measurements were not performed for the test apples.

The approaches for developing prediction models were similar to those used in the previous years. The artificial neural network models were developed relating interactance and fluorescence data to individual maturity parameters. Finally, the combined data of interactance and fluorescence were used in developing the integrated models for predicting apple fruit maturity.

RESULTS AND DISCUSSION

1. The R/LIF and R/UV-F Systems for Measuring After-Storage and Freshly Harvested Apples (Year One/Two)

Table 1 summarizes prediction results for fruit firmness, soluble solids content (SSC), titratable acid, and skin and flesh color (hue and chroma) for after-storage 'Golden Delicious' apples using the R/LIF imaging system for reflectance, fluorescence, and their combined data. Both reflectance and fluorescence correlate with fruit quality parameters. Overall, the reflectance mode is better than fluorescence for predicting apple fruit quality. When reflectance and fluorescence were combined, better predictions of fruit firmness, SSC, and titratable acid were obtained over either reflectance or fluorescence. Skin and flesh color predictions from the combined data are similar to those from the reflectance data. Good to excellent correlations were obtained for all quality parameters except for titratable acid which had low correlation.

Table 1. Prediction results (correlation coefficients) for after-storage 'Golden Delicious' apples obtained with the R/LIF imaging system for three types of data (reflectance, fluorescence, and the combined data) in 2005

Quality Parameter	Reflectance	Fluorescence	Combined Data
Firmness	0.80	0.75	0.86
Soluble Solids	0.72	0.66	0.75
Titratable Acid	0.63	0.57	0.66
Skin Hue	0.97	0.93	0.97
Skin Chroma	0.86	0.78	0.83
Flesh Hue	0.78	0.74	0.76
Flesh Chroma	0.66	0.59	0.70

When the R/UV-F spectroscopic system was used, similar prediction result trends were obtained for after-storage apples of 'Golden Delicious' and 'Red Delicious' (results are not shown here). Again, reflectance is better than fluorescence for predicting individual apple quality parameters, as measured by the coefficient of correlation. When reflectance and fluorescence were combined, prediction results for individual quality parameters in generally improved.

Table 2 summarizes the maturity predictions of freshly harvested 'Golden Delicious' apples obtained with the improved R/LIF imaging system for each sensing mode (i.e., reflectance, fluorescence, and the combined data). For the two single sensing modes, the reflectance mode performed consistently better than the fluorescence mode in predicting all maturity parameters except for flesh hue, in which the two sensing modes had the same results. Predictions for all maturity parameters from the combined data were consistently better than those from either reflectance or fluorescence. The improvements from the combined data over the reflectance data range between 1% and 12% in terms of the correlation coefficient values and up to more than 14% in terms of prediction errors (standard error of prediction). The results for freshly harvested apples in Table 2 also compare favorably to those for after-storage apples (Table 1), although freshly harvested apples are known to

Table 2. Maturity prediction results (correlation coefficients) for freshly harvested ‘Golden Delicious’ apples obtained with the improved hyperspectral reflectance and laser-induced fluorescence (R/LIF) imaging system for three types of data (reflectance, fluorescence, and the combined data) in 2005

Maturity Parameter	Reflectance	Fluorescence	Combined Data
Firmness	0.74	0.63	0.79
Soluble Solids	0.74	0.72	0.78
Starch Pattern Index	0.84	0.70	0.88
Titratable Acid	0.67	0.50	0.75
Skin Hue	0.95	0.94	0.96
Skin Chroma	0.83	0.73	0.87
Flesh Hue	0.90	0.90	0.93
Flesh Chroma	0.66	0.61	0.71

be more difficult to measure over after-storage apples by near-infrared technology. The relatively good results for freshly harvested apples were mainly attributed to the improvements made to the R/LIF system.

Table 3 shows the predictions of maturity parameters for freshly harvested ‘Golden Delicious’ apples obtained with the improved R/UV-F spectroscopic system for the interactance, fluorescence, and combined data. The prediction trends for the R/UV-F system are similar to those for the R/LIF (Table 2). Interactance had consistently better predictions for all maturity parameters than did fluorescence with the exception of skin color, for which the two sensing modes had the same results. Again, the integration of interactance and fluorescence yielded considerably better results than either sensing mode. Relatively low correlation for titratable acid was obtained and this was also observed in the previous study. This indicates the difficulty of achieving accurate predictions of

Table 3. Maturity prediction results (correlation coefficients) for freshly harvested ‘Golden Delicious’ apples obtained with the improved interactance and UV-induced fluorescence (R/UV-F) spectroscopic system for three types of data (interactance, fluorescence, and the combined data) in 2005

Quality Parameter	Interactance	Fluorescence	Combined Data
Firmness	0.75	0.62	0.77
Soluble Solids	0.74	0.67	0.77
Starch Pattern Index	0.82	0.81	0.89
Titratable Acid	0.60	0.53	0.62
Skin Hue	0.97	0.97	0.99
Skin Chroma	0.74	0.74	0.77
Flesh Hue	0.96	0.87	0.96
Flesh Chroma	0.79	0.47	0.77

titratable acid with either sensing mode or the combined data. Relatively poor correlation for titratable acid could also be attributed, in part, to the titration method used, which is prone to experimental error and thus could adversely affect the prediction accuracy by the nondestructive techniques.

Our study and other studies have shown that near-infrared spectroscopy generally has relatively poor predictions of apple fruit firmness and SSC for freshly harvested apples than for apples after storage. This is mainly because freshly harvested apples continue to have high physiological activities immediately after harvest, thus making it more difficult to accurately measure their quality parameters. In addition, the relatively narrow range of firmness and SSC readings for the test apples also contributed to lower correlations. Both reflectance (or interactance) and fluorescence modes had excellent predictions of skin and flesh hue with values for the correlation coefficient of 0.96 and 0.97, respectively, but had lower predictions of skin and flesh chroma (R=0.77 in both). Interactance and fluorescence had similar results for predicting the starch pattern index with values for the correlation coefficient of 0.82 and 0.81, respectively (Table 3). The integrated model had a much better correlation (R=0.89) for starch pattern index prediction.

The results for freshly harvested apples (Tables 2 and 3) are comparable to those for after-storage apples (Table 1). Apparently, improvements to the R/LIF and R/UV-F systems resulted in better maturity predictions for freshly harvested apples.

2. The Laboratory Prototype for Freshly Harvested Apples (Year Three)

The relatively low correlations for SSC and skin and flesh color obtained in the previous were also in part attributed to the narrower spectral range of the spectrometers used. In order to further improve SSC and skin/flesh color predictions, we used two different spectrometers for the prototype, which covered the entire visible range and a greater portion of the near-infrared region (400-1,100 nm). As a result, the performance of the prototype for predicting SSC and fruit skin/flesh color improved. The maturity predictions of 'Golden Delicious' apples tested within 24 hours after harvest with the laboratory prototype are shown in Table 4. Results for starch index and titratable acid are not included in the table. The starch conversion was only observed in a few test apples (which was rather unusual and we still do not know why after consulting with a horticulturist) and thus no starch prediction was performed in the study. Titratable acid measurements have been not been completed yet.

Table 4. Maturity predictions (correlation coefficients) for freshly harvested 'Golden Delicious' apples obtained with the interactance and UV-induced fluorescence prototype from three types of data (interactance, fluorescence, and the combined data) in 2006

Maturity Parameter	Interactance	Fluorescence	Combined Data
Firmness	0.69	0.68	0.74
Soluble Solids	0.89	0.79	0.89
Skin Hue	0.95	0.93	0.97
Skin Chroma	0.80	0.73	0.84
Flesh Hue	0.95	0.90	0.96
Flesh Chroma	0.84	0.87	0.88

The prediction trends for individual maturity parameters obtained with the prototype (Table 4) are again similar to those obtained with the two systems shown in Fig. 1. The prototype had much better predictions of soluble solids and skin/flesh color than the R/LIF and R/UV-F systems (Tables 1-3). The improved results were primarily due to the use of the new miniature visible/NIR spectrometer which had a broader spectral range (400-1100 nm) versus the one (~530-1000 nm) used in the previous systems. The combined data gave better predictions of firmness, skin and flesh hue and chroma, whereas the correlation coefficient for soluble solids from the combined data is the same as that from the interactance data. Firmness predictions from the three data types (interactance, fluorescence, and the combined data) are not as high as those from the previous studies (Tables 1-3). This is mainly because the apple samples from the 2006 harvest season had a narrow range of firmness distributions, thus causing lower correlation.

This research demonstrated that integration of reflectance (or interactance) and fluorescence can lead to improved predictions of fruit maturity parameters, especially for firmness, starch and acid. The integrated technique can provide more consistent and accurate measurement of fruit maturity. The prototype was designed and assembled using two miniature spectrometers, which could facilitate further development of a portable device for field applications. Further improvements in the prototype are needed, especially in selection of appropriate light sources in order to make the prototype truly portable. We will continue our research towards the goal of developing a low cost, portable device for measuring the maturity of fruit on the tree and after harvest.

PUBLICATIONS

Noh, H. K. and R. Lu. 2005. UV/blue light-induced fluorescence for assessing apple maturity. Proceedings of SPIE 5996-19. Bellingham, WA: SPIE.

Noh, H. K. and R. Lu. 2005. Hyperspectral reflectance and fluorescence for assessing apple quality. ASAE Paper No. 053069. St. Joseph, MI: ASABE.

Noh, H. K. and R. Lu. 2006. Hyperspectral laser-induced fluorescence imaging for assessing apple quality. Postharvest Biology and Technology. (In print)

Noh, H. K., Y. Peng, and R. Lu. 2006. Integration of hyperspectral reflectance and laser-induced fluorescence imaging for assessing apple maturity. ASABE Paper No. 066182. St. Joseph, MI: ASABE.

Noh, H. K. and R. Lu. 2006. Integrating fluorescence and interactance measurements to improve apple maturity assessment. Proceedings of SPIE 6381-23. Bellingham, WA: SPIE.

Noh, H. K., Y. Peng, and R. Lu. 2006. Integrating hyperspectral reflectance and fluorescence imaging for assessing apple maturity. Transactions of the ASABE (in review).