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# Fluorescence spectrum analysis on leaf and fruit using the ImageJ software application

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## ABSTRACT

In this study, ImageJ has been used to process fluorescence spectroscopic images of spinach leaf treated with three variations of sunlight. In addition, apples and tomatoes are also used in imaging by treatment immersed in hot water, pierced, and pressed. Leaf and fruits are illuminated by laser diodes and LEDs of different wavelengths. ImageJ is used to calculate the RGB and gray values of the image with two segmentations, namely the intact image and the threshold. The results show that the thresholding method gives the best results because it automatically reduces the image background. In addition, the threshold background can also be easily set in this imaging. For the spinach leaf experiment, LED with a wavelength of 680 nm showed significant differences in each treatment of sunlight intensity. Meanwhile, in the apple and tomato experiment, the diode laser with a wavelength of 405 nm showed significant results. Both types of fruit with this puncture treatment turned out to provide higher intensity than pressed fruit.

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## 1. INTRODUCTION

Fluorescence spectroscopy is a method that can obtain information from a material. This method works to reveal the principle of the interaction of light and matter. Light hitting a material can undergo various processes, one of which is fluorescence. The process of fluorescence occurs when light hitting an object is absorbed, then emitted again [1]. Currently, spectroscopic methods are being developed using charge-coupled device (CCD) cameras or complementary metal oxide semiconductors (CMOS) as detectors. This method came to be called imaging spectroscopy [2]. The results are obtained in the form of images that contain information from the object being observed. The information is in the form of spatial information (the x and y positions of the object), the fluorescence intensity, and the object's wavelength (spectrum) [3].

Image processing program is required to process digital images from CCD and CMOS cameras. This program can be developed using programming languages such as MATLAB and Java. Some image processing programs sold commercially follow imaging systems, especially systems that use cameras [4-6]. These programs are also tailor-made for specific fields such as astronomy and medicine [7, 8]. However, some image processing programs are also available online for free. The image processing program available as open source is imageJ which was developed by the United States Department of Health, but this program needs to be adjusted and calibrated according to its designation [9].

ImageJ is an image analysis program that can display, edit, analyze, process, save, and print color images in grayscale from 8-bit, 16-bit, and 32-bit floating point numbers [10]. ImageJ can also read various image formats including TIFF, PNG, GIF, JPG, BMP, DICOM, FITS as well as raw formats [11]. ImageJ can calculate user defined statistical pixel area. In addition, it can also determine object intensity values and threshold options, so that it can measure distances and angles [12].

ImageJ programs are currently used in various fields of science as image processing software, including biophonics applied to agriculture [13]. Some of the methods that use imaging are fluorescence spectroscopy [14], laser point [15], hyperspectral [16], and computer vision [17]. Fluorescence spectroscopy and laser point imaging are two optical methods used to analyze various aspects of physics in biology and agriculture using a CCD or CMOS camera as a detector and a laser beam as an induction lamp [18]. The interaction of light with biological materials will cause light that hits biological tissue to be reflected, scattered, and transmitted. The light contains information about tissue structure and other characteristics. Images recorded after the material is exposed to light also contain wavelength information (spectral characteristics) [19].

Computer vision and hyperspectral imaging are imaging methods that use polychromatic light as an induced light. Computer vision is one of the image processing methods that is currently being developed and used in the agricultural industry to speed up the process of sorting fruits and vegetables [20]. In this method, the processed image is of biological material exposed to white light. If the desired information is spectral information, this method is called hyperspectral imaging which uses a hyperspectral camera which is commercially available, but relatively expensive [21].

The imageJ program can be used to extract spectrum information from images recorded using cheaper CMOS cameras. ImageJ has been used to classify the quality of tomatoes and lemons based on their physical properties such as size, shape, mass, color, and damage through image processing of fruit exposed to white light [22]. This program is also used to process images of tomatoes containing formalin [23]. ImageJ is a software that can be modified for research needs and can be integrated with other programming languages such as MATLAB for certain applications [24, 25].

In this study, the images analyzed using the ImageJ program were taken from fluorescence spectroscopy for spinach, apple, and tomato leaf. Experiments on spinach leaf images were carried out using fluorescence spectroscopy which was built consisting of two types of light sources, namely LED and laser diodes (LD) with different wavelengths, several optical components, and a CMOS camera as a light detector. Meanwhile, the apple and tomato images were processed using fluorescence spectroscopy and the laser speckle imaging (LSI) system. The images from both experiments were processed using the ImageJ program to obtain the fluorescence intensity in RGB and Gray values. The difference between the two methods lies in taking the image as a whole or only partially through the segmentation process.

## 2. RESEARCH METHODS

This research method was carried out experimentally. In this study, two imaging systems were used, namely fluorescence spectroscopy for spinach leaf and additional LSI for apples and tomatoes.

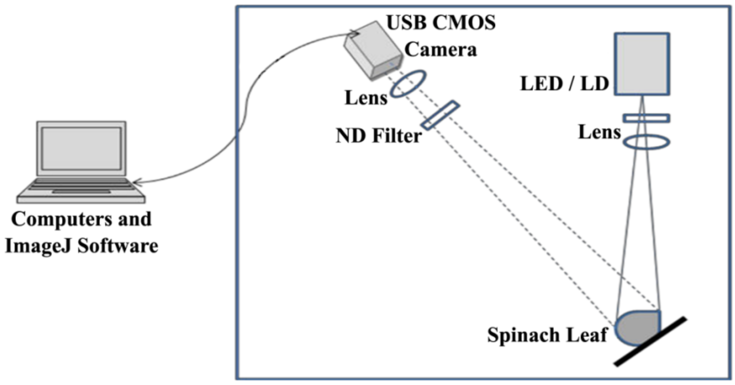


Figure 1. Imaging scheme of spinach leaf using fluorescence spectroscopy.

Figure 1 is an experiment for fluorescence spectroscopy for spinach leaf. The system is built using LEDs, LD light sources, CCD cameras, computer hardware, and ImageJ software. The distance from the light source to the leaf is 28.0 cm, while the distance from the leaf to the camera is 32.3 cm.

The light hits the leaf and the camera forms an angle of 30°. The sample used was spinach which was treated with variations in the intensity of sunlight using plastic and paranet. The 10-day-old spinach plants that were sown by the farmers from the garden were put into polybags filled with soil, given sufficient fertilizer and water, then placed on a plant tent rack that could be opened and closed and installed with a paranet. After the spinach leaf were treated for 10 days (20 days of planting) with plastic covers and paranet, data collection was started. The third leaf from each stem is picked, five of which represent five polybags in each shade, and left for 30 minutes in a black box so that the effects of sunlight are all relaxed by the chlorophyll molecules. The leaf are then placed on black support and illuminated by light from an LED or LD. The wavelength and output power used are 680 nm, 4.5 mW for LED and 780 nm, 20 mW for LD with using a red filter and without a filter. Images containing light fluorescence information are recorded by the image recording software provided by the camera. The saved file is in the form of a BMP file which is then saved by the ImageJ software.

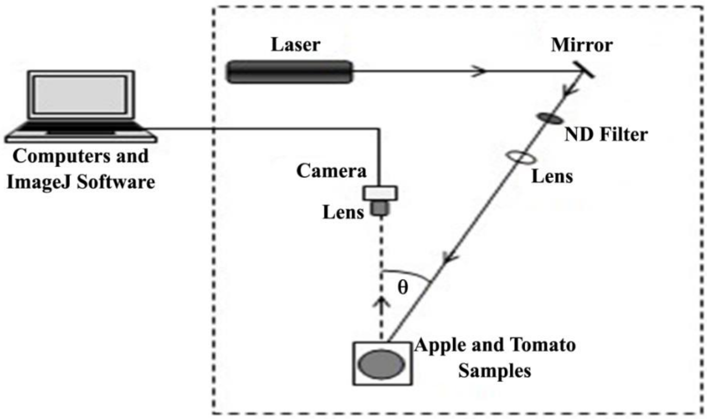


Figure 2. Schematic imaging of apples and tomatoes using fluorescence spectroscopy and LSI.

The second experiment was fluorescence spectroscopy and LSI for apples and tomatoes as shown in Figure 2. The fluorescence spectroscopy method uses a red and purple LD with wavelengths of 650 nm and 405 nm, a power supply for a diode laser, a 3MP CMOS camera equipped with image camera software, a camera lens with a focal length of 35 mm and a maximum aperture of f/20, filters, lenses, mirrors, and ImageJ image processing software. While the LSI method uses a He-Ne laser with a wavelength of 632.8 nm. The samples used in both methods are the same, namely apples and tomatoes, both of which are green. Tomato samples were treated in the form of pressure, puncture, and hot water immersion. Image processing for the results of the second method is done using ImageJ software. The fluorescence spectroscopy method will display the color intensity diagram (RGB value) and the LSI method will display the Gray value diagram. The last method is done by using the entire image and thresholding, namely removing the background (background from the image) by setting the threshold value.

## 3. RESULTS AND DISCUSSIONS

Image data that is processed using ImageJ is taken from two experiments. Figure 3 shows the shape of the image and the results that were processed using the ImageJ program. The first experiment used the fluorescence spectroscopy method for spinach leaf. The data in this study were spinach leaf from plants that were given 3 treatments of sunlight intensity, namely without paranet (I, 90%), one paranet (II, 40%), and two layers of paranet (III, 32%). The light source used is 680 nm LED, 780 nm LD, red filter, and no filter in front of the CMOS camera.

Images from the first experiment were processed into three categories. The three categories are RGB image data that is processed as a whole, RGB image data that is processed using thresholding, and converted to 8 bits (grey) to get a gray value. The results of leaf image processing for the treatment of variations in solar intensity using a 780 nm laser diode, 680 nm LED, filter, and without a red filter. The red filter serves to filter out the wavelengths detected in the red area.

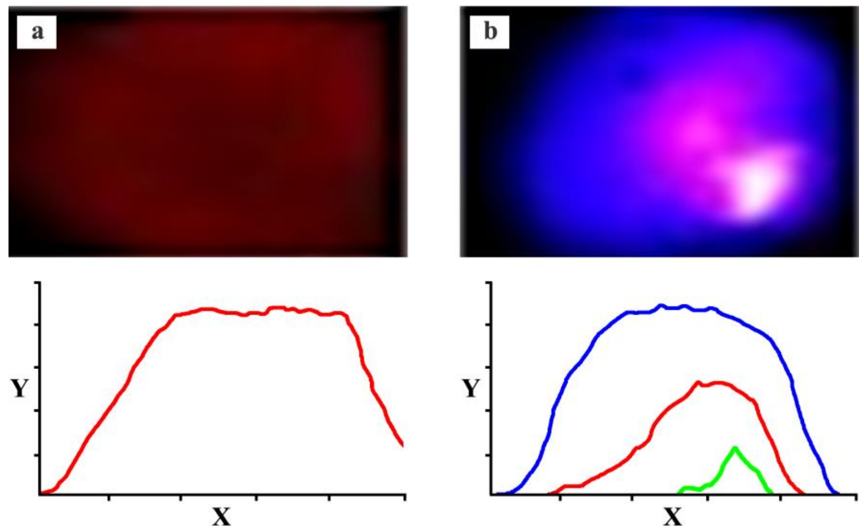


Figure 3. RGB intensity for the entire image as a region of interest (ROI) of (a) spinach leaf are irradiated with a red laser and (b) fruit (apple and tomatoes) are irradiated with a purple laser (right).

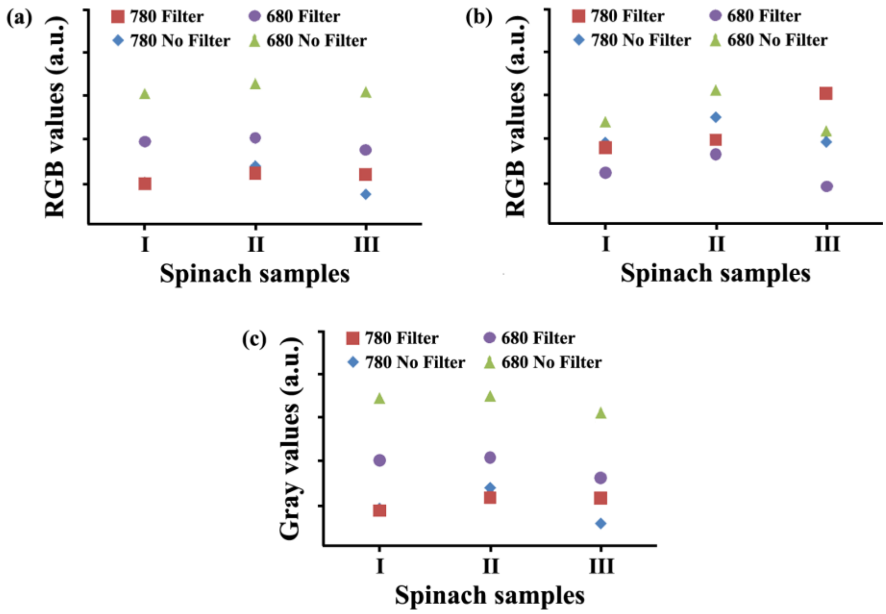


Figure 4. Image processing results using ImageJ for spinach leaf using RGB values with (a) all images as ROI area, (b) RGB image with ROI thresholding area, and (c) conversion of RGB to Gray with ROI thresholding area.

Figure 4 (a) is the result of ImageJ image processing with overall image ROI. The predominant RGB intensity comes from the leaf illuminated by an unfiltered 680 nm LED light followed by a red filter. The highest intensity was obtained on spinach leaf which were treated with 32% sunlight intensity. But overall, the results did not show a significant difference for the three treatments. Figure 4 (b) is the result of image processing using the thresholding method. The results are different from Figure 4 (a). The background is the area around the object that can affect the RGB intensity or the calculated Gray value. In Figures 4 (a) and 4 (b) the threshold is not set to zero or at a certain threshold value, this causes the calculation standard deviation value to be greater than the average value or in other words, the RGB and Gray mean values are low. In Figure 4(b), the intensity values for the unfiltered and filtered diode lasers are higher. Thresholding is a method that directly reduces the background value. Figure 4 (c) is the result of image processing after being converted to 8 bits (grey), then the value of the gray level is determined. The results are not much different from the fluorescence intensity using the RGB component with an overall regional ROI.

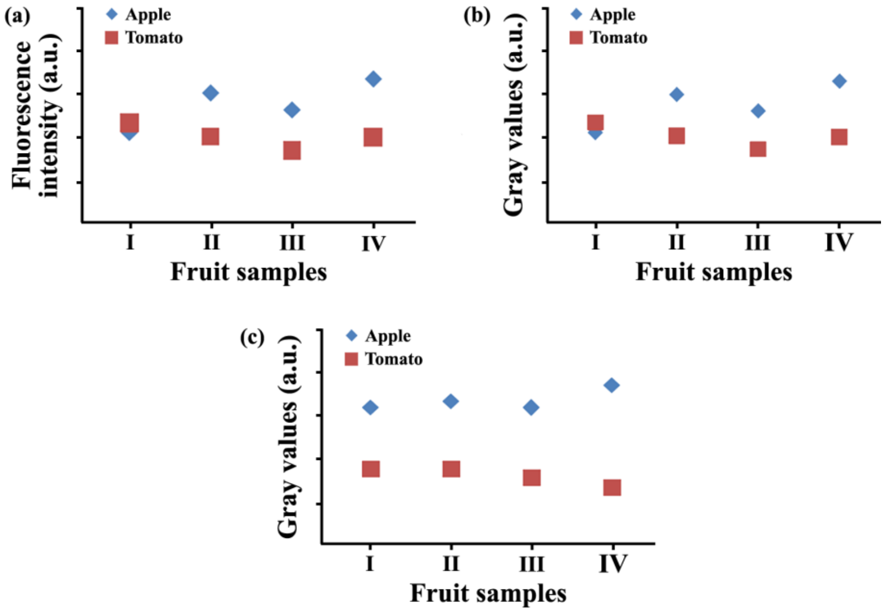


Figure 5. Image processing results using ImageJ for tomatoes and apples using RGB values with (a) all images as ROI area, (b) Gray image with thresholding area ROI, and (c) Gray with significant thresholding as ROI area.

Figure 5 (a) is the result of processing the fluorescence spectroscopy image for processed tomatoes and apples and their RGB values representing the fluorescence intensity for the overall ROI of the image. The threshold method is difficult to do for color images. From Figure 5 (a), higher fluorescence intensities are provided by the 405 nm diode laser except for untreated apples. Figure 5 (b) is the result of fruit image processing with treatment on tomatoes and apples after the color image is changed to Gray. For the overall ROI, the image gives identical results to the RGB values in Figure 5(a). While Figure 5 (c) which uses thresholding provides a significant distance for the use of a 405 nm laser. Overall, the 405 nm laser gave high RGB and Gray values, and skewered tomatoes gave higher values followed by pressed tomatoes.

## 4. CONCLUSION

Image processing using the ROI area method has been successfully carried out. This research was conducted in several ways, namely overall and using thresholding from leaf and fruit images. Each gives a different result. The use of thresholding gives a better value because it can automatically reduce the background image. For the leaf experiment, the 680 nm LED gave a clear difference between the three solar intensity treatments. For fruit, overall the 405 nm laser provides high RGB and Gray values. Skewered tomatoes scored higher than pressed tomatoes.

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