ELECTRO-MICROSCOPE BASED 3D PLANT CELL IMAGE PROCESSING METHOD

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ABSTRACT

Agricultural products are easily deformable its shape because of some external forces. However, these force behavior is difficult to measure quantitatively. Until now, many researches on the mechanical property was performed with various methods such as material testing, chemical analysis and non-destructive methods. In order to investigate force behavior on the cellular unit of agricultural products, electro-microscope based 3D image processing method will contribute to analysis of plant cells behavior. Before image measurement of plant cells, plant sample was cut off cross-sectioned area in a size of almost 300-400 μ m units using the micron thickness device, and some of preprocessing procedure was performed with fixing and dyeing. However, the wall structure of plant cell is closely neighbor each other, it is necessary to separate its boundary pixel. Therefore, image merging and shrinking algorithm was adopted to avoid disconnection. After then, boundary pixel was traced through thinning algorithm. Each image from the electro-microscope has a information of x,y position and its height along the z axis cross sectioned image plane. 3D image was constructed using the continuous image combination. Major feature was acquired from a fault image and measured area, thickness of cell wall, shape and unit cell volume. The shape of plant cell was consist of multiple facet shape. Through this measured information, it is possible to construct for structure shape of unit plant cell. This micro unit image processing techniques will contribute to the filed of agricultural mechanical property and will use to construct unit cell model of each agricultural products and information of boundary will use for finite element analysis on unit cell image.

Keywords: Cellular Mechanics, Electro-microscope, 3D Plant Cell, Agricultural and Mechanical Property, Finite Element Analysis

INTRODUCTION

The research related on the physical and mechanical property of the agricultural products was mainly studied through the manner of material testing and force behavior analysis through the numerical methods such as finite element analysis(FEA).

Agricultural products consist of unit cell, and it has a some regular structure such that cell nuclear, wall of cell and free water between the cell. The cell structure of each plant shows a different multiple facet shape and this shape contains a many useful information on the physical property. Soft cell tissue in agricultural products such as fruits and vegetables is highly susceptible to mechanical damage during harvesting, handling, transport and storage. This damage is caused by external loadings and is manifested a irreversible change in the structure, color and taste of the tissue. An important area of research has been the relation between the externally applied loads and deflections, and the internal damage it induce in the cellular structure of the materials(Pitt,"1982"). In present, many research on the nondestructive quality evaluation technique such as computer vision, ultrasonic, X-ray and NMR technique was introduced on the fields of physical property. Many research was perform with various approaching methods. Some research on the physical property was perform with cell structure via FEA and material testing(Pitt(5-8)).

And model cell based material testing was performed with rubber material, and performed analysis with finite element method(Umeda, "1994",Motomura,"1995"). Through this research, it will be contribute to the analysis on the handling mechanism of agriculture products such as manipulator of harvest machine, packaging device and shape and other related handling machinery.

The goal of this research was a measurement for microscope image and development of processing algorithm to collects a 3D plant cell information. Through this information, force behavior and modeling for cell structure will perform with FEA.

METHODS AND MATERIALS

To observe the cell image structure via electron microscope, preparation procedure of sample plant is important. There are various methods for preparation procedure to get a clear image, the acquired image would differ according to the treatment reagent and fluorescence solution. In image processing, complex algorithm was needed such as image separation, enhancement, skeleton and 3D image processing technique because of the each acquired cell image shows a shape having a connected boundary between cell walls. The measurement software was developed using the MFC library of Microsoft Visual C++ 6.0 as shown in fig. 7-(a) and the detailed preparation procedure was illustrated in Fig. 1.

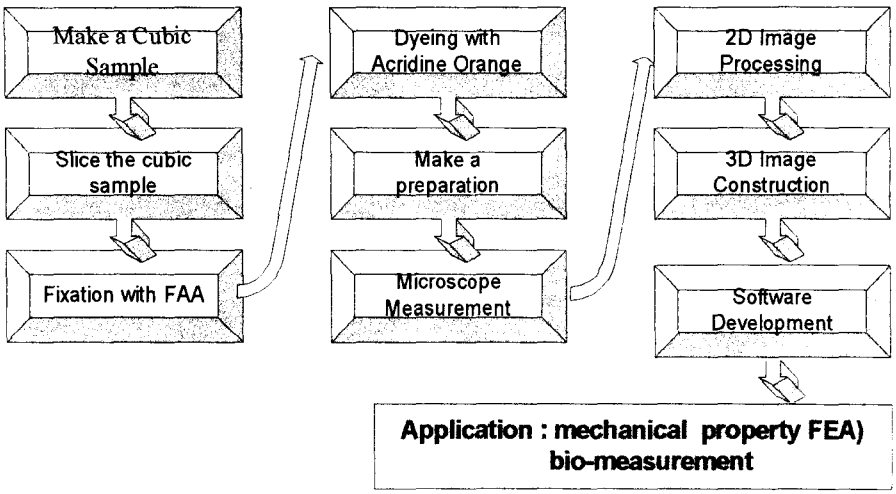


Fig.1 plant cell image measurement procedure.

Permanent preparation with slide and cover glass of sample

The electron microscope was used Olympus Co. FLUOVIEW system of fluorescence scanning electron microscope(SEM) and microscope is BX-50 model. This system has a standard laser lines for blue and green, single 488nm line Argon ion laser(blue excitation) and dual 488/568nm line Kripton-Argon line laser(blue & green excitation) can use of it. In image detection, simultaneous 2 channel fluorescence or combination fluorescence/DIC detection is available. The continuous image measurement along the Z-axis can available by automatic focusing and image acquisition(Z-sectioning) via high precision z-axis stepping motor.

The permanent preparation of observation sample is important and needed well trained operation technique. The procedure of the laser SEM has complex preprocessing steps before image analysis. General preparation procedure is divided into fixation, dehydration and dyeing(coloring) with fluorescence reagent as denoted in Fig. 1.

Before fixation processing, sample was prepared for cubic shape as Fig. 2, and the size is almost 8x8x5(mm^3). This cubic samples was sliced with 300μm height unit using the micron slice device. This micron slice device was operated by step motor and sample slice hydrate with a distilled water, and the slicing depth of device can control amount of 0.1mm unit. Using the sliced thin sample, moisture of this sample must eliminated and deposited into the cell fixation solution. The purpose of fixation treatment is prevent for serious degeneration and can maintain the original plant cell structure. In this experiment, FAA(formaldehyde:5ml, acetic acid:5ml, ethanol: 45ml, distilled water: 45ml) solution was used to fix the prepared sample and the fixation time takes 1 hour. After fixation, sample must do

dehydration processing through the ethanol 85% solution get rid of the lens deformation that affect to reflectance rate, and dehydration time is 1 hour. Because of the laser Algon gas's wavelength is 488nm and fluorescence solution for dying(coloring) also has a same activation wavelength, acridine orange has a 490 nm response wavelength and it was used to dye plant sample.

To make a permanent preparation set consist of slide and cover glass, put the sample on the slide glass and deposit with immersion oil on the sample, and then put the cover glass on the slide glass. This procedure was denoted in Fig.2. The edge part of the cover glass was sealed with special glue prevent for deformation of sample cell. The role of immersion oil can prevent for reflectance characteristics according to the lens. When observing with microscope, immersion oil must be deposited on the cover glass and between the slide and cover glass.

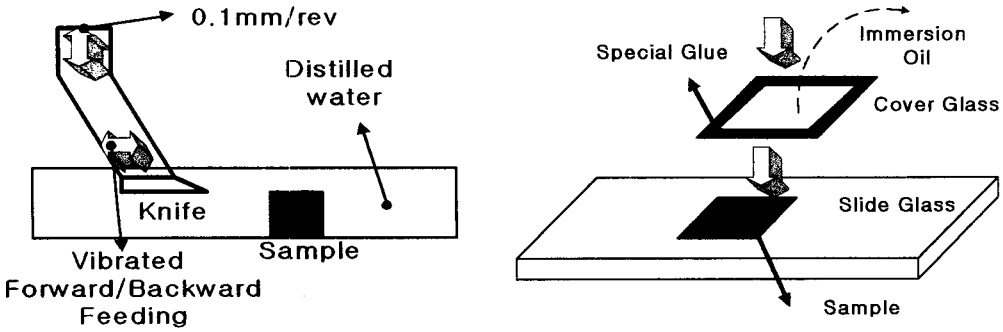


Fig. 2 Micron Slicing Device and preparation with slide and cover glass.

Image measurement and processing

After preparation, image measurement through laser SEM was performed and image was stored to memory device. And BX50 microscope is possible to control step motor and its maximum resolution is 0.1μm, it is possible to know the sample thickness about the motor revolution along the z-axis. The experimental flowchart was shown in Fig. 3 and ch1 is a transmittance mode.

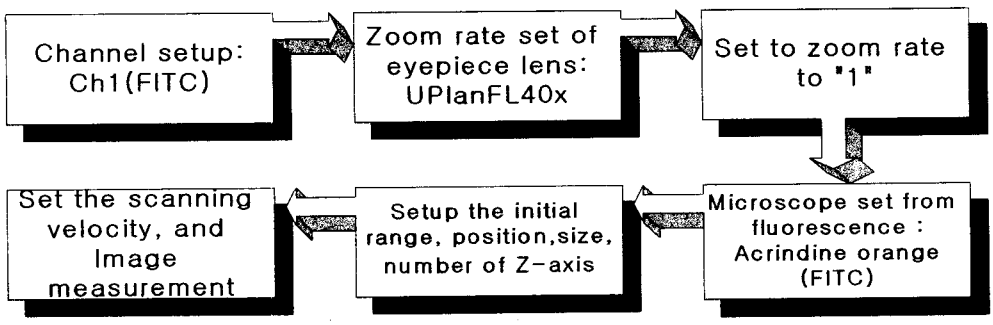


Fig. 3 laser SEM operation structure

Image processing algorithm and software was developed using the BMP format image from the laser SEM system. Each image set was defined unique number along the measuring Z-axis. The shape of original cell image was showed combination of connected circle, basic image processing algorithm was adapted to get a unit cell's image. Because of the fluorescence color is similar with red, thresholding was determined by red color band, and mode separation method was used to find a threshold value automatically. The binary image has a bimodal histogram such that inside of the cell wall area are all colored with unique value and cell wall has a different value. Next time, morphological image erosion algorithm was adapted to avoid of the connecting effect about inside area of the cell wall. Erosion is the morphological transformation that combines two sets by using containments as its basis set. If A and B are sets in Euclidean N-space, then the erosion of A by B is set of all element x for which $x+b \in A$ for every $b \in B$. The erosion of A by B is denoted by $A \ominus B$ and defined as below formula and do refer the detailed algorithm of erosion(Robert,"1992").

$$A \ominus B = \{ x \in E^N \mid x+b \in A \text{ for every } b \in B \}$$

When cell wall is connected each other, the measurement for cell boundary coordinate information is really difficult through automatic manner by the bad recognition of unique cell image. If it is not to be sure, another recognition or mode separation algorithm must require to find whether it is one or more objects.

Prewitt template matching method was used to find an edge coordinate it is similar with basic pattern matching methods(ITII,"1994"). To avoid noise effect, moving averaging method also adapted after erosion processing. Based on the searching pixel, all value of 3x3 mask was added, and then averaged pixel value P_{av} can calculated that was divided by total processing element number. X is a current pixel intensity of each mask element(ITII,"1994").

$$P_{av} = \sum_{i=0}^{8} Xi / 9$$

Edge detection and noise reduced image have a some pixel thickness. To avoid it, image thinning algorithm was adapted(Hilditch,"1969"). Each boundary coordinate information of the cell image is important to design the mesh generation of finite element analysis(FEA). Chain coding algorithm is useful to apply previous comment, but entire cell image has a shape of multiple objects. Therefore, image labeling was needed for distinguish to each cell objects and algorithm is simple. Based on the current pixel position P, scanning mask was defined as neighbor 4 pixel coordinate such as left-up direction is Q, upward direction is R, right-upward direction is S and left direction is T. If the intensity of P has a 0(background),going on scanning, and check the Q,R,S,T coordinate when P is 1(object). The latter case was divided into three state such that if Q,R,S and,T are all zero then assign a new label into

the current pixel P, assign a label to P for that one of them is 1 and assign one of the labels to p that two or more neighbors pixel are 1. At the end of scan, all points with value 1 are labeled. Sort all pairs of equivalent labels into equivalent classes. assign a unique label to each class rescan through the image, replace each label by the label assigned to its equivalent class.

The size of cell wall was measured by scanning from centroid point of object 1 to another object cell as shown in fig. 4.

Fig. 4 Cell wall finding between multiple cell image

RESULTS AND DISCUSSION

Above mentioned image processing algorithm was tested, and software was developed by Microsoft visual C 6.0. Through the experiment, preparation of sample is important to acquire clear cell image such that treatment time is so short or late, acquired result was not showed normal image. In fig.5 shows that the preparation condition affluence to the image acquisition, (a) is broken image and dark part illustrated for deformation of cell by the heavy load when the cover glass was fixed on the slide glass.

(a)broken image of apple (dyeing: 30 minute)

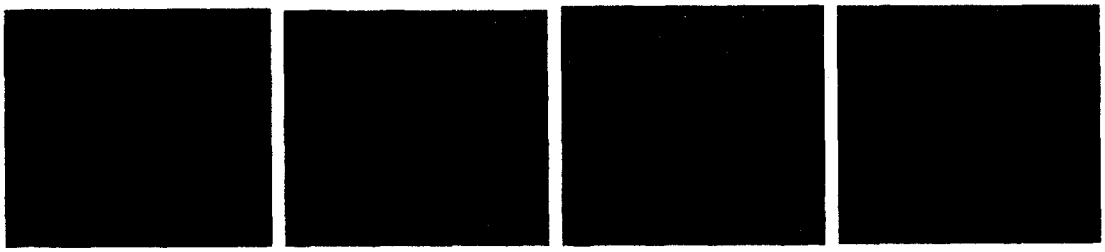
(b) broken image of apple (dyeing :5 hour)

(c) normal image of watermelon (dyeing: 1 hour)

Fig. 5 Image from the laser SEM.

Watermelon was used to the sample plant, and above explained algorithm was adapted. After thresholding via mode separation method was performed and then,edge detection, noise reduction, thinning and multiple cell image measurement was also done. Chain coding algorithm added with real value converting variable can possible to calculate the quantitative analysis in measuring stage(Lee,"1995"). Although this software is included real value converting variable, the shape and orientation information such as boundary coordinate, centroid and distinguished label among multiple object is more important in order to apply the cell modeling with FEA. Mesh generation of FEA will be more easily performed with continuous measured along the Z-axis using whole boundary information.

The processing result image of watermelon was showed in the fig. 6. By the erosion, binary image converted into isolated objects, fig 6-© show the feature extraction through Prewitt operation and thinning.



(a)original (b)binary (c) averaging after erosion (d) thinning after Prewitt

Fig.6 The result image of step by step processing

The constructed 3D image was shown in Fig. 7, the measured image set from the laser SEM was 45 and step motor's step size was 3μm. Each image set was measured the boundary information and it will be use to the input data of mesh generation for FEA and 3D feature generation. In order to get a more approximate 3D modeling of all agricultural products, curve fitting methods will guarantee for more reliable results.

Further research will perform the approximation of model for 3D plant cell through above mention algorithm, and will do finite element analysis through the real experiment of material testing such as compression and rheology characteristics. 3D plant cell analysis via finite element methods is a interesting research approach about physical and mechanical property in comparison with previous material testing via part sampler. It has a problem about real measurement of loading force for cell and statistical verification of modelized cell structure having a various shape. However, this kind of analysis methods will gives a more scientific problem solving and it will be contribute on the fields of all handling device for agricultural products and food such as packaging design, manipulator design and sorting device etc..

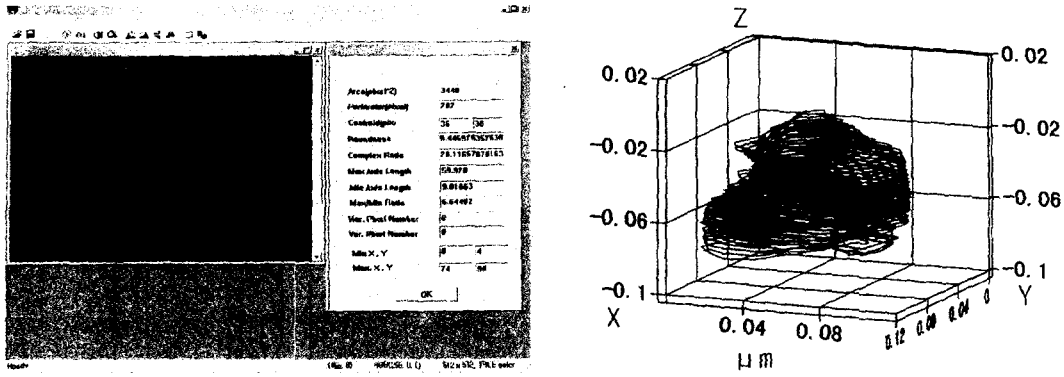


Fig. 7 Software tools developed and constructed 3D image.

CONCLUSIONS

This research was mainly performed on the feature extraction methods and preparation method for microscope operation. Plane image set was constructed to 3D structure through step by step image processing. The real size of cell can measured by real scaled variables via chain coding and multiple cell was continuously measured in a single plane image.

Through this research, plant cell analysis will be perform with FEA and material testing. However, the modeling of approximated standard cell is difficult because of the its random complex shape caused by the sample condition such as moisture content, storage periods, and other environmental factor. Therefore, it is must consider for analysis on the mutual relationship between cell, cell wall, affluence from the free water and inner pressure. Finally, it will be adapt to the optimum design for agricultural and food handling mechanism through the loading force analysis via FEA.

Also, this microscope based measurement technique will contributed on the precision measurement, modeling and analysis for the bio-resources.

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