

*Full length article***THREE-DIMENSIONAL RECONSTRUCTION AND VISUALIZATION OF PLANT CELLS**Sharifa zaman^{1,2} B. Fatima¹

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ABSTRACT

The mechanical properties (like sensory texture etc.) of plants/fruits directly depend on their microstructures. Therefore, it is very important to well understand the geometry and topology of cells in order to control the microstructure for better mechanical response. In this research, techniques of digital image processing and segmentation in conjunction with mathematical morphology models are used to visualize and analyze the 3D cells of potato. ImageJ and MATLAB are used throughout in this study. The labeled image stacks are essential for studying quantitative characterization of 3D cells, MATLAB is used to label each image stacks. By using MATLAB 12420 cells were segmented within a short period of time and labeled each cell uniquely.

KEYWORDS: Mechanical properties; Cellular structure; image processing; visualization

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1. INTRODUCTION

Nature is composed of three-dimensional structures. We observe different materials and objects in our daily life that contain microstructure which is composed of cell/grains, which are called cellular structures. The properties of materials strongly depend on their microstructure. Biological science and engineering are also influenced by the cellular structure, it is found in biological tissues and foams. The most important topological properties of cells are their shape and size, which play a vital role in evolution of microstructure [1].

Cell is a building block of living thing, which is extremely composite having different shape and size. To study cellular properties its spatial association of the cell is most important than that of epigenetic conditions [2].

To study the geometrical and topological properties of cells, microscopy techniques play a vital role. A complete explanation of cellular structure needs the knowledge of mechanical and geometrical characteristics of each cell, but this is not an easy task to know the pure characteristics of an irregular arrangement of cells, therefore volume and shape of individual cell is needed[3]. There are many techniques which are used to visualize and characterize the cellular structure in 3D like Tomography (X-rays or Ultrasound) and stereology [4].

In the case of plants, we study the cellular structure at considerable non-homogeneity and instability with numerous areas of discontinuity of relatively large dimensions. The morphology of plant structure is a derivative of number of factors, which are plant variety, size of object and shape, type of tissue and techniques of storage situations [5].

The mathematical study of properties that are conserved by deformation, stretching's, twisting's of objects is known as topology. By using Voronoi construction, the study of the topological properties of system can be achieved, which gives a division of the entire space in irregular panels (cells). Such process has been fruitfully used to describe the evolution of many natural systems. Such as propagation of defects in foams, soap froths and biological tissue cells. All these works are fundamentally mean-field studies and only interpretation for correlation between adjacent cells, numerous topological relations discussed in the past, containing Aboav-Weaire law on nearest neighbor correlations [6, 7] an growth rate of single cells[8], the Lewis law is based on the statistics of cell area and the scaling law is based on distribution of the cells, the Aboav-Weaire law is an essential empirical manifestation to define the short-range topological correlations between a center grain and its first nearest neighbors in two dimensions,

$m_1(n) = A_1 + B_1/n$. Here m_1 is the average number of edges of first nearest neighbor, $A_1=5$

and $B_1=6+u_2$. A large dataset of the topology of cells and their nearest neighbor is needed to study long-range topological properties [9].

Visualization of 3D microscopic images help us to recognize the data clearly. It also helps to find suitable analysis techniques or parameters.

The 3D microstructure representation, visualization and correlation are of most necessary concern to study and model processing structure property interaction; most frequently due to the 3D nature and complexity of material microstructure, studies are usually carried out in 2D image sections through 3D

microstructural region of interest using stereological methods. These stereological methods have been very useful in studying the progress of microstructure and provide important insights into the microstructural characterization. Nevertheless, the connectivity or topology of cells in microstructure cannot be studied by using the methods of stereology, and there are many other factors like true size, shape and curvature of interfaces that needs the understanding of 3D microstructure. Thus, 3D reconstruction and visualization of microstructures are very important to obtained true 3D morphology of cells.

There are many methods for the representation of 3D microstructure as well as serial sectioning, magnetic resonance imaging and X-ray tomography [10]. Serial sectioning is a commonly used method in microstructural visualization, which can attain approximately all the information concerning 3D geometry of cells in cellular structure. Image processing and segmentation techniques play a vital role in micro structural visualization and analysis. Latest advancements in imaging techniques have significantly reduced the efforts required to perform 3D analysis and made it possible to computerize these processes to some extent. The objective of this contribution is to explain an approach to apply automated image processing and segmentation for 3D visualization, reconstruction and analysis of 3D cell microstructure. The image processing and analysis software NIH ImageJ [11] and MATLAB are used for such performances.

Grew felt that plant cells needed to study in 3D because they were composed of microscopic structure having distinct shape and size, he first worked on reconstructing vascular element (Xylem and phylum) in three dimensions by using depth and perspective to his illustrations,

and deal with the technological limitations of visualizing the inner part of opaque 3D tissues. Grew's illustrations gave us the first indications of how varied and complex the cellular structure of plants. To understand the relationships between xylem structure and its function is a challenge of visualization of 3D structure with traditional, two dimensional methods. In the time of Grew photography and histological methods were invented, which are used in visualizing and reproduction of xylem networks compared with manual illustration. To reconstruct the path that water passed through roots and stems, number of serial sections are needed, then stacked the sections and these stacks must be perfectly aligned to visualized and reconstruct the xylem network manually, which is tedious task [12].

In this paper, advanced digital image processing techniques in combination of mathematical morphology models are used to reconstruct and visualize 3D cellular structure of potato.

2. MATERIALS AND METHODS

2.1 Plant material

Plant cell are composed of microscopic structure with different spatial relationships. To better understand the cellular structure of cell, it needs to be visualized and modeled in 3D. Therefore, potato about 5×10^4 um diameter was chosen for sampling. The sample was cut into square shape of 2×10^4 um in diameter. Only images of this region of potato were chosen for study. Since the purpose of this research study was to visualize the cellular structure of plant cell (cells of potato).

2.2 Microtome

Microtome is a device used to cut extremely thin slices of material, called sections. Microtomes are used in microscopic studies, allowing for the preparation of samples for observation under transmitted light or electron radiation for further studies.

In this study we used microtome (Model MRS Cat No 213, Nippon Optical Works Co.LTD) for serial sections of potato. The sample was 2×10^4 um in diameter, were cut into 28 serial sections of thickness 714.286 um.

2.3 Optical microscope with integrated digital camera

Optical microscope is an instrument invented by Dutch Optician and Van Jansen in 1595. It is very simple device for generation of magnified images of material or object which were not seen by naked eyes [5]. In this research study optical microscope was used for observation of serial sections with magnification ratio of (10x/18) and objective lens of (10x/0.25). Sections were imaged using a (Sone) digital camera of 20 mega pixel.

2.4 Image processing software's (IMAGEJ and MATLAB)

To visualize the internal cellular structure of potato cells in a suitable way digital image processing and segmentation techniques in combination with mathematical Morphology Models are used. Image processing software's MATLAB and IMAGEJ are used in this research study. These software's are easy to used and understand, are very effortless to carry out various steps of visualization. The detailed steps are as under.

Firstly, the images were imported into ImageJ and then set the measurement scale. For this

purpose, we used a stack of 28 images of potato cell.

The alignment of 28 images, to remove the Translational and rotational shift by using plugin, during performing the steps of visualization which may affects the stacks.

To get the region of interest the stack of aligned images was cropped in a square shaped.

Circular and rectangular shape stack are also available but square and rectangular shaped stack was more optimal. The researcher used this ROI to segment the 2D images.

For clean up the dust present in every 2D images various segmentation processes (adjusting the brightness or contrast, dilation, Erosion, thresholding, watershed, distance map and finding maxima) were used. For binary images apply threshold on an image, because of this binary image got noise in the objects which makes the images not clear.

Dilate and erode are used to remove the noise, because of the dilation and erosion noise on the boundaries of cell get clean and clear. Watershed is used for filling the gaps and separates the overlapping particles. To find local maxima of images Euclidean distance map is used. Selecting segmented particles from dropdown menu to creates one segmented particle per maximum.

A stack of 28 segmented images having very clear and clean boundaries and neat cells, which were labeled by using MATLAB program.

3. RESULTS AND DISCUSSIONS

The 3D visualization and correlation of 28 images of potato cell is the 3D quantitative analysis of the microstructure; we studied the cellular structure of cell without disturbing its true morphology. For quantitative analysis of cell, firstly convert the serial sections of images into 3

dimensional stacks. There are several steps of image processing and segmentation to visualize the cellular structure and to study morphology of cells. These are as under.

3.1 Importing of images in to image J

The first step is to import images into ImageJ, initially we open image J window and put all images in a folder by a proper manner. This can be done by browsing through *file>import>image sequence*. Then stack the images, the stack contains 28 images which we used throughout in the research study. Shown in fig. 1

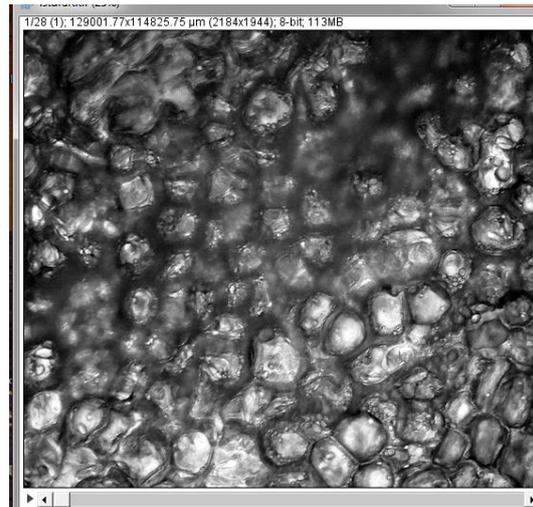


Fig.1 The stack of 28 images

3.2 Alignment of images (image registration)

Second step is registration of images, which was done by over lapping two consecutive images manually translating and rotating the images with respect to each other. The second image with respect to first and third image with respect to second and continued the process until all images are registered with respect to each other by applying *Plugins>Align stacks>Align3TP*. See in fig.3 the alignment was done in few minutes and got a perfectly aligned

stack as a result. To show the true feature of microstructure, during the serial sectioning of image its need to remove translational and rotational shift by using alignment of images.

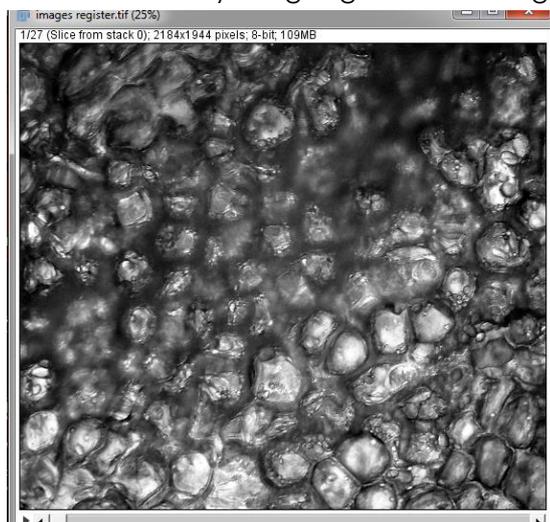


Fig.2 aligned stack.

3.3 Segmentation and 3D Reconstruction of images

Third step is image cleanup and segmentation. It is an important step to analyze the cells without difficulty. The main objective of image segmentation is to separate the object of interest from background. There are many types of image segmentation software's but the most reliable and preferable software's in every field like soil science, astronomy, biological sciences is ImageJ. ImageJ is easy to use and easily available which is used by every person from every field of life, because it gives the quantitative results without disturbing the true morphology of cells in a very short period of time. The several methods used for segmentation and image cleanup include methods like thresholding, watershed segmentation, region growing, edges detection and so on are used to segment each two-dimensional image in a stack to view the cell in three dimensions to get desired quantities. The

detail of following steps which are used in image segmentation are

3.3.1 Cropping image

Before doing segmentation process, first to select the region of interest (ROI), this was done by selecting the rectangular bar from the menu bar of ImageJ and cropped the desired area by using *image>cropped*.

3.3.2 Thresholding

After cropping the region of interest, the researcher needs to find the boundaries of cells and separation from background.

First convert the images into a binary stack by applying a threshold command using *Image>adjust>threshold*. This command gives black color to pixels in background and while interior of cell is considered white.

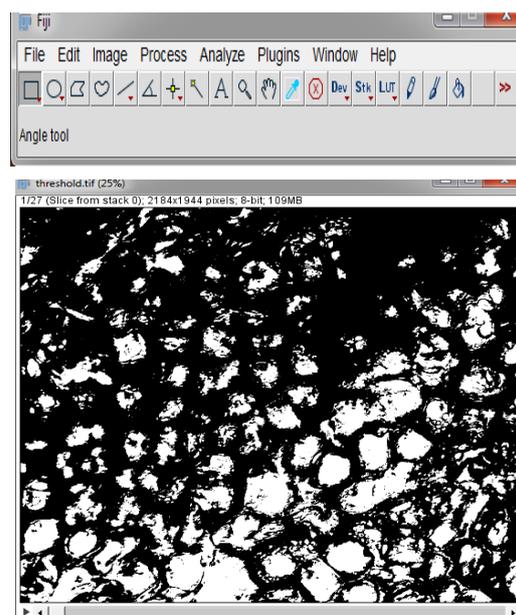


Fig.3 cropped stack.

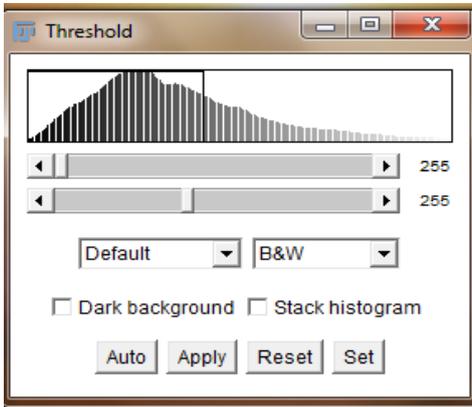


Fig.4 Thresholding process in imageJ

3.3.3 Morphological operations

By using ImageJ, we applied some morphological operations to clean up the spots which were left inside the cell after threshold. The morphological operations are erosion, dilation, watershed and Euclidean distance map. These commands can be reached by *process > Binary > Dilate* and *process > binary > Erode* are used to clean up the image stack, due to Thresholding and morphological opening some boundaries of cells are unfilled and some cells overlap due to low contrast among them, therefore 3D watershed segmentation is considered to be a best choice to filled these gaps, which is reached by *process>binary>watershed* command from menu.

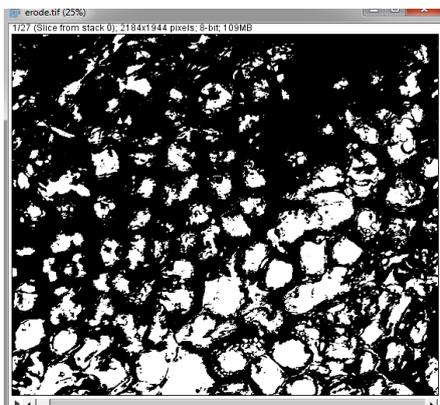


Fig.5 Dilation of images

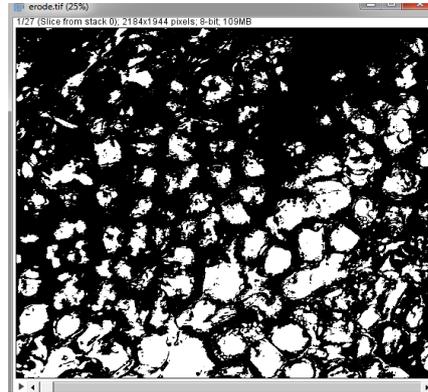


Fig.6 Erosion operation on images

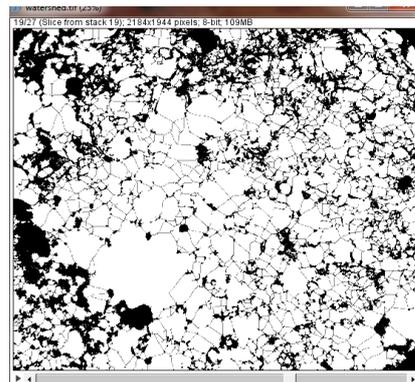


Fig.7 Watershed segmentation

3.3.4 Euclidean distance map and finding maxima

Create a Euclidean Distance map by selecting *process>Binary>distance map* and to find maxima reached by *process>find maxima*.as shown in fig.8

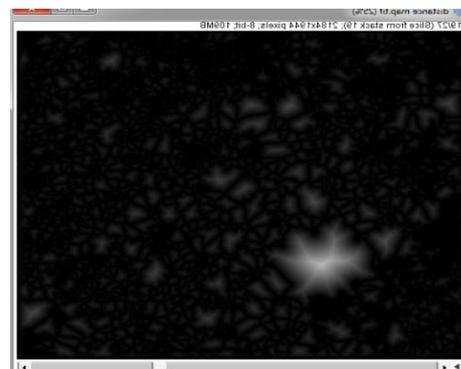


Fig.8 Euclidean distance map of images

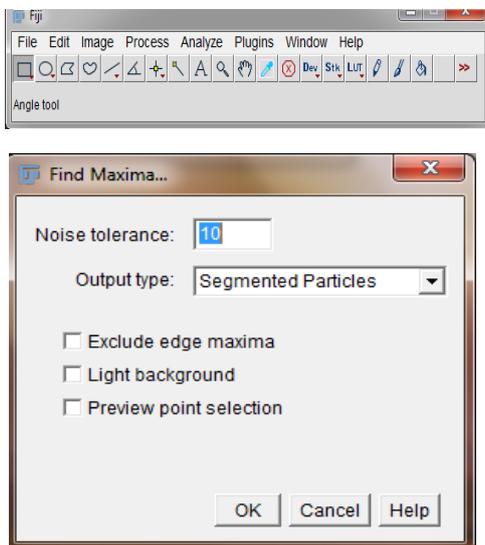


Fig.9 The Euclidean distance map of images

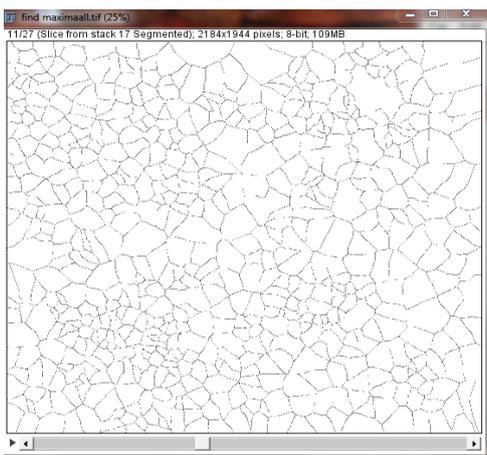


Fig.10 Result of maxima operation

3.4 Three Dimension Visualization labeling method

For labeling 2D images, *analyze>Analyze particle* command are used. It can be applied to 3D image stacks but in result it labels every particle independently and gives different identifiers to

Same cells, to avoid this 3D object counter is used by research plugin> 3D object counter, but in this research 2D images analysis is used because of irregular shape of cells of images stacks. In this research MATLAB is used to label each images stack.3D microstructure are visualizing to better comprehend the data and

it helps for appropriate analysis methods or parameter. Furthermore, it is also useful to study the grain morphology and for understanding connectivity of microstructural place. Once the image stack is scaled correctly, it is ready for visualization

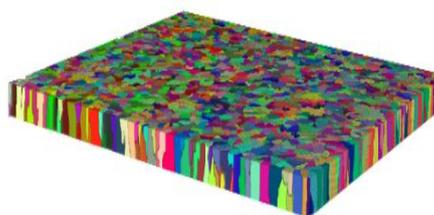


Fig.11 Labeling of 28 images stack and image stack in 3D



Fig.12 Labeled image stack in 3D

CONCLUSION

The mechanical properties (like sensory texture etc.) of plants/fruits directly depend on their

microstructures. Therefore, it is very important to well understand the 3D geometry and topology of cells in order to control the microstructure for better mechanical response. In this paper, techniques of digital image processing and segmentation in conjunction with mathematical morphology models are used to visualize and analyze the 3D cells of potato. ImageJ and MATLAB are used throughout in the study. The labeled image stacks are essential for studying quantitative characterization of 3D cells and MATLAB is used to label each image stacks. By using MATLAB 12420 cells were segmented within a short period of time and labeled each cell uniquely.

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