Int. Agrophysics, 2007, 21, 49-53

INTERNATIONAL
Agrophysics
www.ipan.lublin.pl/int-agrophysics

Analysis of plant tissue images obtained by confocal tandem scanning reflected light microscope

M. Gancarz, K. Konstankiewicz, K. Pawlak, and A. Zdunek*

Institute of Agrophysics, Polish Academy of Sciences, ul. Doświadczalna 4, P.O. Box 201, 20-290 Lublin, Poland

Received September 25, 2006; accepted December 1, 2006

A b s t r a c t. The paper presents two methods (automatic and semi-automatic) for quantitative evaluation of cell structural parameters of plant tissues. The methods were developed for images obtained by confocal tandem scanning reflected light microscope. Quality of the images is sufficient for semi-automatic analysis. However, the automatic method does not give satisfactory results because it gives mean cell area 30% bigger and 60% fewer cells than in the semi-automatic method. Therefore, we state that for images taken by confocal tandem scanning reflected light microscope semi-automatic analysis is more accurate and proper at this moment.

K e y w o r d s: structural parameters of plant tissue, image analysis, confocal microscope

INTRODUCTION

Highly developed technologies require more and more knowledge of material properties. The above is also true of agricultural raw materials used both for direct consumption and industrial processing (Wilkinson *et al*., 2000). Structure is one of the most important properties of the material, directly associated with other properties of the material centre. Studies have shown that, among others, the microstructure influences the mechanical resistance of plant tissues (Pitt and Chen, 1983; Zdunek and Konstankiewicz, 2004; Fornal *et al*., 2000) which undergoe changes during drying (Wang and Brennan, 1995), freezing (Da-Wen Sun and Bing Li, 2003) and also as a result of heating (Aguilera *et al*., 2001).

To show the complexity of plant tissue structures, microscopic images obtained by various techniques are used. However, most often such structures are evaluated descriptively, and it is only possible to utilize structural studies when the structure is described numerically (Kalab *et al*., 1995; Pukos, 1994; Pukos *et al*., 1995; Fornal *et al*., 1999). In order to describe the structure of a plant tissue, especially its changes as a result of all kinds of impacts, it is necessary to carry out observations preserving the most natural state possible of the object studied. Microscopic methods usually require complex procedures of preliminary sample preparation and should take into account any structural changes at this stage of the examination (Konstankiewicz, 2002; Konstankiewicz *et al*., 2001; Petran *et al*., 1995). The most frequent microscopic images of the structure are flat cross-sections. Quantitative analysis of such images is limited to determination of the geometrical parameters of the structural elements and their location in relation to one another. The lack of universal methods and computer procedures which could be applied for various types of materials is a serious obstacle to this type of study (Cwajna *et al*., 1994; Konstankiewicz *et al*., 1998; 2001; 2002).

The aim of this study was to develop a procedure of analysis of images obtained by confocal microscope. In the paper we will compare the results of analysis obtained by automatic and semi-automatic method.

MATERIALS AND METHODS

The inner core of potato tuber parenchyma, cultivar Danusia, was chosen in order to elaborate the method of analysis (Fig. 1). It has hardly any starch content, vascular bundles and intercellular spaces within the tissue. Therefore, this simple structure is very useful for testing methods. Potatoes came from the harvest of 2003, grown at the Department of Potato Storage and Processing of the Institute of Plant Breeding and Acclimatisation in Jadwisin.

*Corresponding author's e-mail: marko@demeter.ipan.lublin.pl

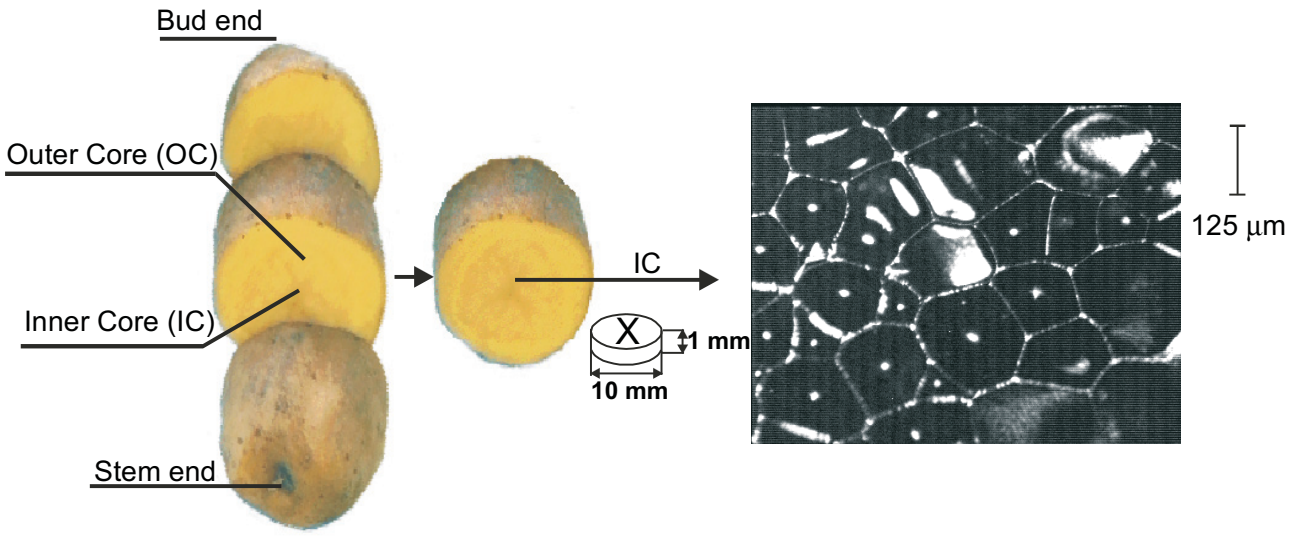

Fig. 1. Sampling place (inner core) within potato tuber and example of microstructure image.

A slice of 1 mm in thickness and 10 mm in diameter was cut from the central part of the potato tuber, from the inner core, by means of a special guillotine cutter equipped with two parallel blades (Fig. 1).

Immediately after cutting, the sample was rinsed in distilled water in order to remove starch and other cell components that potentially remained on the surface. Next, the slice was mounted on a microscopy slide and gently drained off with filter paper. Samples prepared in such a way were then subjected to observation by means of an optical confocal microscope (Tandem Scanning Reflected Light Microscope - TSRLM) (Petran *et al*., 1995). A-plan 10/0.24 lenses were used for the present observations and the images were taken by a digital camera with resolution of 752 x 582 pixels in grey scale of 0-255. This allowed observation of 10-15 whole cells in one image. The linear dimensions of the image were 0.82 x 0.65 mm. In this experiment, 50 non-overlapping images were taken.

RESULTS AND DISCUSSION

The procedure described above was developed in order to obtain cell structure that would be easy for automatic analysis of 50 images in a single procedure. As a result, obtained images have high contrast (Fig. 2). The cells are clearly visible as polygons with thin walls. Unfortunately, some of the walls are not continuous, which may make the analysis difficult. Additionally, inside each cell there are other shining objects (they can be bottoms of the cells or remains of water after tissue washing) that may also influence the result of the analysis.

The aim of the analysis was to obtain the area and the perimeter of each cell separately. All 50 images were

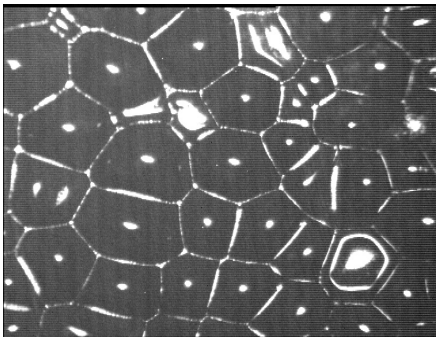

Fig. 2. Microscope image of potato tuber tissue.

analysed in two ways: 1) automatic, where the images were processed in a computer automatically, and 2) semi-automatical, where the walls within the images were first manually sketched and next the sketches were measured. The second way of analysis is a reference method, because, in most cases, it is easy for the observer to recognize the cells even if the wall is broken.

Automatic analysis

The main steps of automatic analysis are shown in Fig. 3a – d. As a tool for writing the procedure, Aphelion software was used. The procedure consists of a set of morphological operators. The operators:

– enhance the images *ie* link the walls, delete objects that are not cells,
– convert images to binary format,
– recognize the cells,
– measure the cells.

The aim of this part of the procedure (erosion, reconstruction, dilation and opening operators, Fig. 3a) was to extract big and bright objects that were not cells. As a result, the big and bright objects from Fig. 3a were deleted (Fig. 3b). The transformation allowed extracting objects (areas within the image) that in the next step lain were 'sources' of the cells (Fig. 3c). The border cells were deleted from further analysis (Fig. 3d).

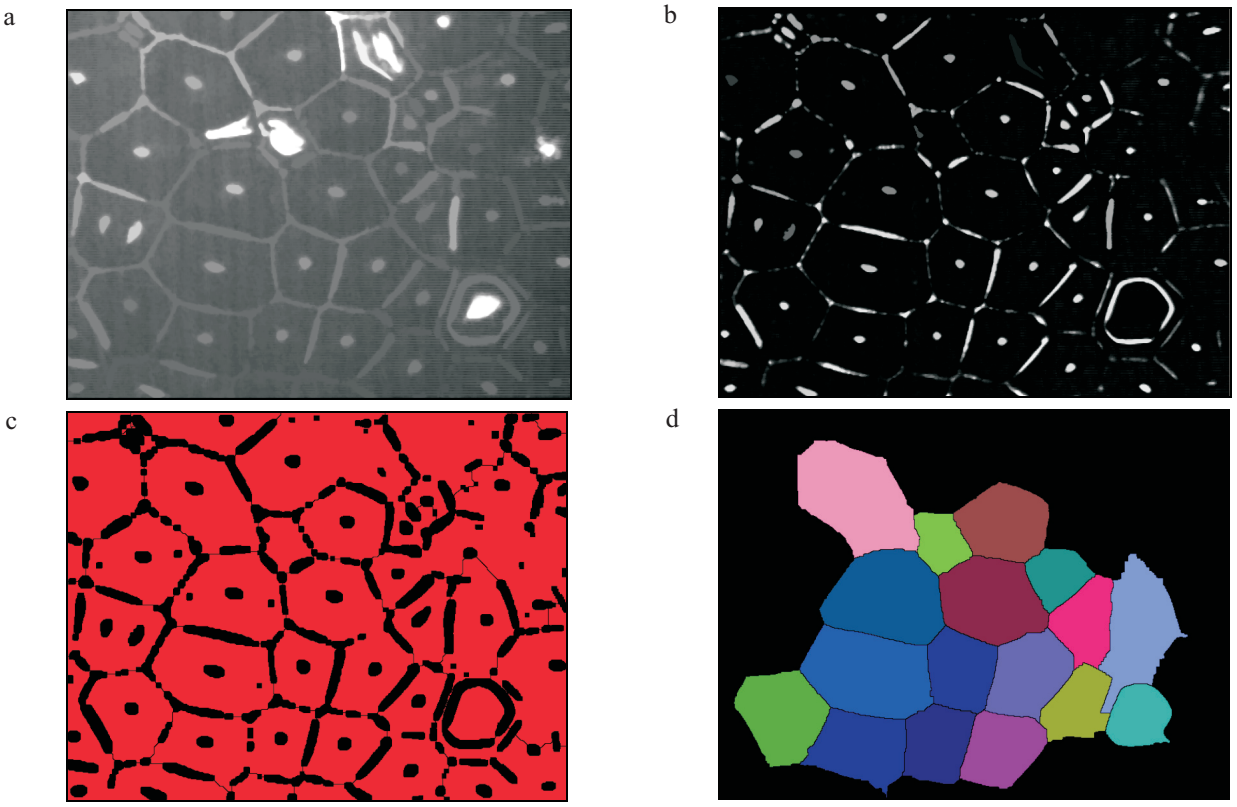

Fig. 3. a – result of erosion, reconstruction, dilation and opening operators; b – difference between source image and the image form the Fig. 3a; c – binarisation and transformation into convex areas using the distance function; d – the watershed operator finds and labels the cells.

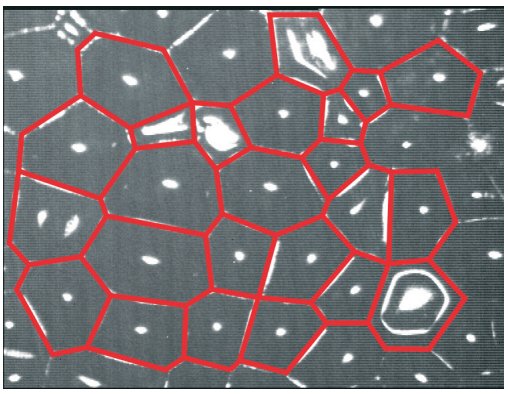

Fig. 4. Source image and manual sketching of the cell structure. The red lines were drawn on a separate layer.

Semi-automatic analysis

In order to check the quality of automatic analysis, the semi-automatic method was used as the reference method. Sections representing individual cell walls were drawn on the background of the original image by means of Corel Draw. The sketches were drawn on a separate layer, as straight segments from the corner to corner of the cells. In that way, a skeleton of the structure in the form of closed polynomials representing 'two-dimensional' cells of plant tissues were obtained, as shown in Fig. 4. The binary sketches were then processed by watershed operator that detects and labels the cells. Similarly to the automatic procedure, the border cells were deleted. Next, each cell was measured.

RESULTS

In order to compare the methods of analysis, two geometrical parameters were determined: the area and the perimeter of each cell. A comparison of the results is shown in Table 1. Significant differences in the results can be observed. The size of objects (mean area and perimeter) obtained by method 1 is higher than in method 2. The difference is about 30%. However, the number of detected

T a b l e 1. Comparison of two methods of analysis of potato tuber tissue images obtained by TRSLM

Parameters of cell	Automatic analysis		Semi-automatic analysis	
	Mean value	SD*	Mean value	SD
Area (μm^2)	17813	9441	12011	6134
Perimeter (μm)	712	223	521	138
Number of detected objects	426		1049	

*Standard deviation.

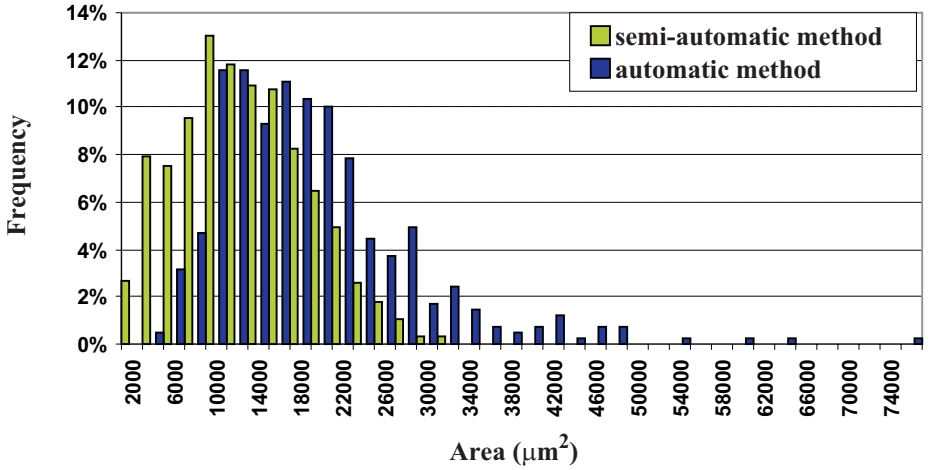

Fig. 5. Distributions of cell area obtained by automatic and semi-automatic methods.

objects is lower. In the automatic method we lost about 60% of the cells. In Fig. 5 the exact cell area distribution is presented. It is seen that the distribution obtained by the reference method is moved to the left. However, there are no significant differences in the range around the peaks. The biggest differences appear on the tails of the distributions.

In the automatic method, the same computer procedure is applied for all images. However, individual features of the images cause different errors of reconstruction. Typical errors are: losing some cells, linking two or more cells in one object and detecting objects within the real cell as walls. These errors cause higher values of area and perimeter in comparison to semi-automatic (reference) method 2. In the semi-automatic method the result depends on the observer. However, breaks of the walls and white objects within the cells do not influence the observer's decision on the reconstruction. Quality of the images is sufficient for assessing a sketch of the cell walls almost within the whole observation area. On the other hand, the automatic method does not give satisfactory results because the difference of 30% in area and 60% in number of cells is significant. Therefore, we state that for images taken by confocal tandem scanning reflected light microscope semi-automatic analysis is more accurate and proper at this moment.

CONLUSIONS

1. Quality of images taken by confocal tandem scanning reflected light microscope is sufficient for unambiguous recognition of cells.

2. The automatic analysis developed in this study does not give satisfactory results because of broken cells and other disturbing objects within the images.

3. Accurate analysis requires manual sketching of the cell structure.

REFERENCES

Aguilera J.M., Cadoche L., Lopez C., and Gutierrez G., 2001. Micro-structural change of potato cells and starch granules heated in oil. Foot Res. Int., 34, 939-947.

Cwajna J., Szala J., and Maliński M., 1994. Image processing and image analysis in materials science: atlas-part I. Proc. Int. Conf. 'Stereology and Image Analysis in Materials Science' - STERMAT'94, October 3-6,1994, Wisła, Poland.

Da-Wen Sun and Bing Li, 2003. Micro-structural change of potato tissues frozen by ultrasound-assisted immersion freezing. J. Food Eng., 57, 337-345.

Fornal J., Jeliński T., Sadowska J., and Quattrucci E., 1999. Comparison of endosperm microstructure of wheat and durum wheat using digital image analysis. Int. Agrophysics, 13, 215-220.

Fornal J., Sadowska J., Ornowski A., Jeliński T., and Velikanov L., 2000. Damage resistance and microstructure of barley kernels. Int. Agrophysics, 14, 159-166.

Kalab M., Allan-Wojtas P., and Miller S.S., 1995. Microscopy and other imaging techniques in food structure analysis. Trends in Food Sci. Technol., 6, 177-186.

Konstankiewicz K., 2002. Determination of geometrical parameters of plant tissue cellular structure (in Polish). Acta Agrophysica, 72, 61-78.

Konstankiewicz K., Czachor H., Gancarz M., Król A., Pawlak K., and Zdunek A., 2002. Cell structural parameters of potato tuber tissue. Int. Agrophysics, 16, 119-127.

Konstankiewicz K., Guc A., and Stoczkowska B., 1998. Determination of the structure parameters of potato tuber tissue using specialistic image analysis program. Pol. J. Food Nutr. Sci., 7/48, 3, 59-64.

Konstankiewicz K., Pawlak K., and Zdunek A., 2001. Quantitative method for determining cell structural parameters of plant tissues. Int. Agrophysics, 15, 161-164.

Petran M., Hadrawsky M., and Boyde A., 1995. The tandem scanning reflected light microscope. Int. Agrophysics, 9, 275-286.

Pitt R.E. and Chen H.L., 1983. Time-dependent aspects of the strength and rheology of vegetative tissue. Transactions of the ASAE, 26(4), 1275-1280.

Pukos A., 1994. Qantitative description of structural changes in soil and plant materials during deformation. Int. Agrophysics, 8, 103-112, 1994.

Pukos A., Pezda M., and Guc A., 1995. Digital analysis and potato tissue image processing at the application of Voronoi's diagrams. Int. Agrophysics, 9, 319-328.

Wang N. and Brennan J.G., 1995. Changes in structure, density and porosity of the potato during dehydration. J. Food Eng., 24, 61-76.

Wilkinson C., Dijksterhuis G.B., and Minekus M., 2000. From food structure to texture. Trends in Food Sci. Technol., 11, 442-450.

Zdunek A. and Konstankiewicz K., 2004. Acoustic emission in investigation of plant tissue micro-cracking. Transactions of the ASAE, 47(4), 1171-1177.