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Visual texture analysis for cell size measurements from confocal images**

A. Zdunek[1]*, *R. Konskyy*[2], *J. Cybulska*[1], *K. Konstankiewicz*[1], *and M. Umeda*[3]

[1]Institute of Agrophysics Polish Academy of Sciences, Doświadczalna 4, 20-290 Lublin 27, Poland
[2]Karpenko Physico-Mechanical Institute, National Academy of Sciences of Ukraine,
Naukova 5, Lviv 79601, Ukraine
[3]Laboratory of Field Robotics, Division of Environmental Science and Technology, Graduate School of Agriculture,
Sakyo-ku, Kyoto 606-8502, Japan

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A b s t r a c t. Methods of obtaining images and their quantitative analysis should be developed in order to analyse an influence of properties of cellular skeleton on mechanical properties of a tissue.

The paper presents visual texture analysis method for quantitative measurements a mean cell size from confocal images of potato and carrot tissue. The confocal images were obtained using the confocal laser scanning microscope (CSLM) and segmented using procedure developed. The accurate mean cell size obtained after segmentation was compared with a size of the structural element of a closing morphological operator applied to the same images. It was obtained that visual texture analysis allows obtaining quantitative information about the size of the cells within images obtained by the CSLM. The quantitative information about the mean cell size within the confocal image can be obtained by both calculating an inflection point on $G(i)$ curve or by a grey level variation $g(i)$ analysis. Common recalculation equation for obtaining the mean cell size can be used for both materials potato and carrot.

K e y w o r d s: image analysis, confocal microscope, segmentation, visual texture analysis

INTRODUCTION

Fruits and vegetables are built mainly of thin walled parenchyma cells highly susceptible to mechanical damage. Mechanical properties of these soft plant tissues depend on an external loading (Bajema *et al.*, 1981) and set of material properties, like turgor (Pitt, 1982; Pitt and Chen, 1983) and variety (Konstankiewicz *et al.*, 2001a). One of the least known, but now intensively investigated, is an influence of cells geometrical parameters on a tissue mechanical properties.

In order to analyse an influence of properties of cellular skeleton on mechanical properties of a tissue, methods of obtaining images and their quantitative analysis should be developed. A few attempts were done for this purpose. This is obvious that microscopes have to be used to obtain images of the cellular structure. Among many different types microscopes available the the confocal microscopes seem to be the most useful.

Konstankiewicz *et al.* (2001b) have used the confocal microscope called Tandem Scanning Reflected Light Microscope. According to obtain images for analysis a special method was used in that research. Thin 1 mm slices were cut and washed in a water to remove residues from the surface. Next, 2D images of potato tissue in natural state were taken. Since some objects within the cells still existed, thus images were outlined by hand over the walls and next automatically processed in order to obtain geometrical parameters of cells and their statistical distributions. This method gives satisfactory results however the outlining requires much time and high attention. Better results would be obtained using confocal scanning laser microscope CSLM. Zdunek *et al.* (2004) have developed a simple sample preparation protocol and an image analysis procedure to obtain automatically geometrical parameters of the cells from 2D image. In this research, from not fixated tissue slice

*Corresponding author's e-mail: a.zdunek@ipan.lublin.pl
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10 images in unbiased way were taken and image segmentation into cells was performed. The method provide accurate measurements of each cell within the confocal images, however if some object is not segmented correctly, manual correction is possible also. Segmentation of the confocal images is usually much easier than images obtained by other types of microscopes. This allows very precise analysis of size and shape distribution to extract certain type of tissue from the image. This was done by Guillemin *et al.* (2004) who developed procedure for the automatic clustering of plant cells observed by the confocal microscopy allowing revealing histological regions. In this research confocal images were merged to obtain bigger mosaic image where different histological regions were visible. In spite of this that the segmentation of the confocal images is relatively effective comparing to other micro- scopes, new methods of image analysis are still looking for due to make analysis faster and cheaper.

In this paper a visual texture analysis method (VTA) is proposed for the confocal images. The VTA was proposed by Devaux *et al.* (2005) for images obtained by device for macro-imaging developed. For these images the segmentation cannot be applied due to small image resolution. The VTA bases on the grey level analysis of an image after application to them a morphological operator with increasing size of a structural element. In result, a maximum at grey level gradient between images corresponds to a size of objects in the source image.

The aim of this work is developing and testing a new efficient method based on the visual texture analysis to 2D confocal images of potato and carrot tissue in order to obtain quantitative information about a mean size of cells.

MATERIAL AND METHODS

Potato tubers (*cv. Barei-so*) and carrot roots (*cv. New Kuroda*), purchased as one batch from a local grocery store in Japan, were examined in the experiment. The material was not selected with respect to size and shape. The material was stored in plastic bags in a refrigerator for 1-2 days, at a temperature of around 6°C, and then was brought to room conditions for 24 h before the test. The experiment was carried out on cylindrical samples. Potato samples were taken from the centre of the tuber *ie* the pith core. Carrot samples were taken from the inner part of the root, next to vascular bundles. Cylindrical sample (7 mm in diameter by more than 7 mm long) was glued to the table of a micro-roto-slicer (D.S.K., DTK-1000, Dosaka, Japan). Three slices (0.5 mm in thickness for potato and 0.3 mm for carrot) from the top of the sample were cut off by razor blade for microscopic observation.

In order to obtain images of the cell structure of the tissues, a CSLM (Olympus Fluoview, Olympus Corporation, Tokyo, Japan) was used. The procedure of sample preparation and obtaining images was described by Zdunek *et al.* (2004). Briefly, slices of the tissues were stained in aqueous Coriphosphine O solution (excitation wavelength 460 nm, emission wavelength 575 nm) for 10 s and next washed in tap water for about 10 s. Immediately after this, the samples were mounted on a microscope slide and carefully drained off by tissue paper that caused its sticking to the slide as well. An argon-ion laser (450-515 nm) was used in this experiment. The observation utilized an Uplan FI 10X/0.30Ph1 lens. Images recorded in tagged information file (TIF) format had resolutions of 512×512 pixels. One image covered 1.96 mm^2 (1.4 mm×1.4 mm). All settings: confocal aperture, magnification, saturation, contrast, filtering, *etc* were the same for all images taken both for potato and for carrot. From one tissue slice, 20 overlapping images were taken. Next, they were merged together to get mosaic images of size about 5x5 mm. Within one image there were about 2500 cells. Totally, 50 mosaic images of potato and 30 mosaic images of carrot were analyzed.

Next, the images were analyzed by means the same computer procedure as developed by Zdunek *et al.* (2004). The procedure used a set of morphological operators: DilateReconClose, Skeleton and Watershead (Aphelion, ADCIS, Hérouville Saint-Clair, France) that improved the images and segments the image into cells. After segmentation the area of each cell was calculated and next the mean value from each image were obtained by the procedure.

Visual texture analysis

Visual texture analysis procedure bases on method developed by Devaux *et al*. (2005). The method of visual texture analysis was realized using Aphelion (ADCIS, Hérouville Saint-Clair, France) software, where a special procedure was founded. The procedure consists of the following steps:

1. Read image and measure its total grey level $G(0)$,
2. Choose type of the structural element. In order to obtain cell size a square structural element has been chosen. If elongation or orientation of the cells is interested, a linear structural element can be chosen for example,
3. Apply closing morphological operator to a source image with a size of the structural element i x i, where $i=1,2,....n$ is the number of pixels,
4. Measure the total grey level $G(i)$ of the resulting image.
5. Export $G(i)$ to a calculation chart and calculate the grey level variation according to the formula:

$$g(i)=\frac{G(i)-G(i-1)}{G(0)} \quad (1)$$

The steps 3-5 are repeated with increasing the size of the structural element i until the total grey level $G(i)$ is equal 1 (the image become white).

RESULTS AND DISCUSSION

In the experiment, 50 images of potatoes and 30 images carrot obtained by CSLM were selected. The criterion was having a wide range of cell size within images analyzed. Thus, segmentation of more 1000 mosaic images was performed firstly and a mean value of cell size from each image was calculated. An example of segmentation of the mosaic images is presented in Fig. 1. From this large bath of mean cell size values, 50 images of potato and 30 images of carrot were chosen due to have a broad representation of cell sizes for each material; for potato, images with the mean cell size from 6 798 to 18 151 μm^2; for carrot, images with the mean cell size from 4 853 to 11 337 μm^2.

An example of results of applying the closing operator with increasing size of the structural element to potato and carrot images is shown in Fig. 2. Applying increasing size of the structural element causes bolding of the white element (cell walls) within the image and gradual disappearing of the cells. For potato, applying closing operator with the size of the structural element of 16x16 pixels and for carrot applying closing operator with the size of the structural element of 9x9 pixels causes the highest change of the grey level. Figure 3 shows variation of the total grey level *G* in a function of the size of the structural element *i* for an exemplary image of potato and carrot, the same as shown in Fig. 2. The curves have third-order polynomial character. At the given above sizes of the structural element (mentioned at Fig. 2)

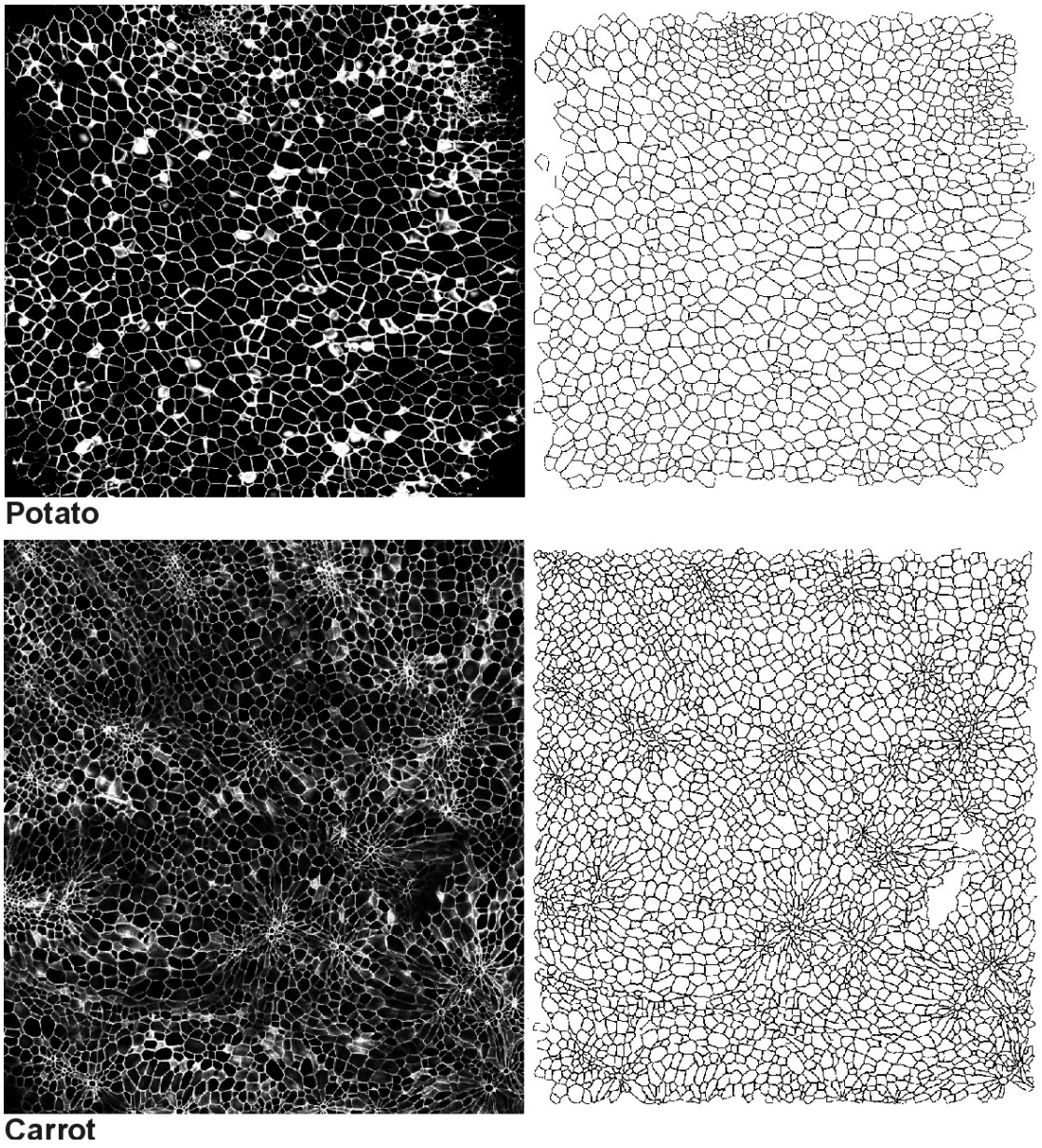


Fig. 1. Example of segmentation (to the right) performed on potato and carrot confocal mosaic images (to the left).

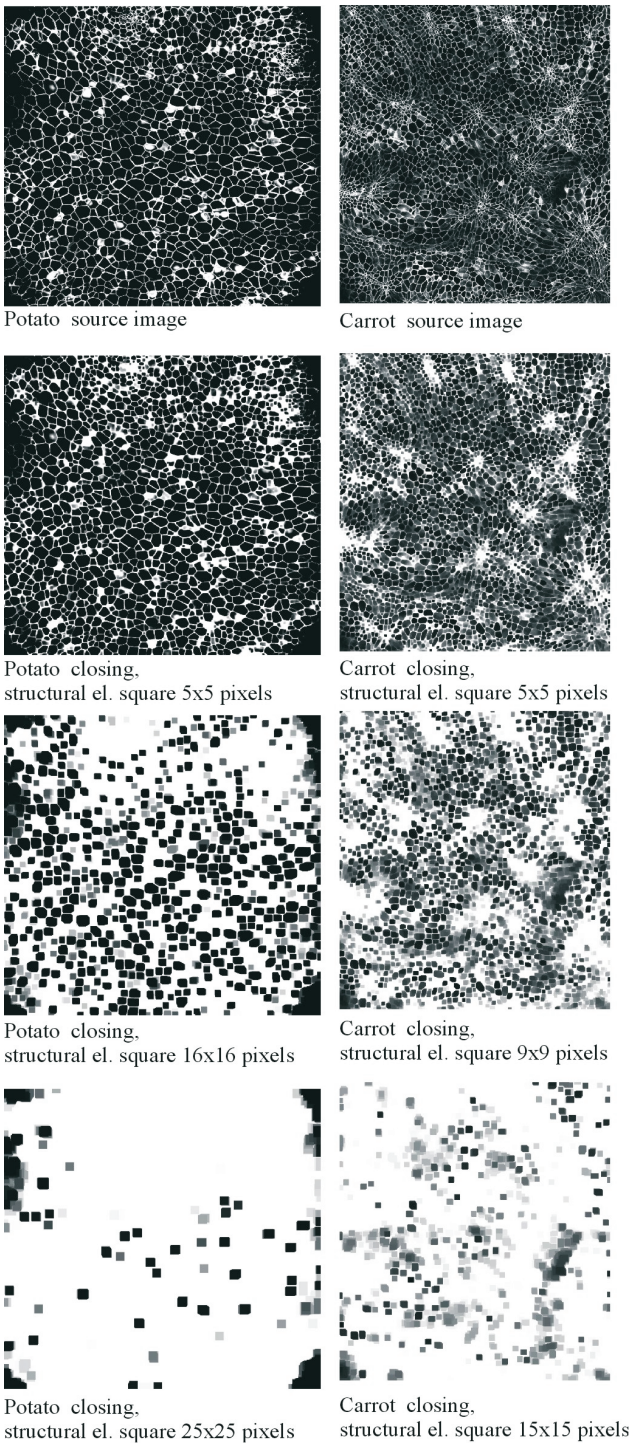


Fig. 2. Results of acting of closing morphological operator with increasing size of the structural element for potato (to the left) and for carrot (to the right) confocal images.

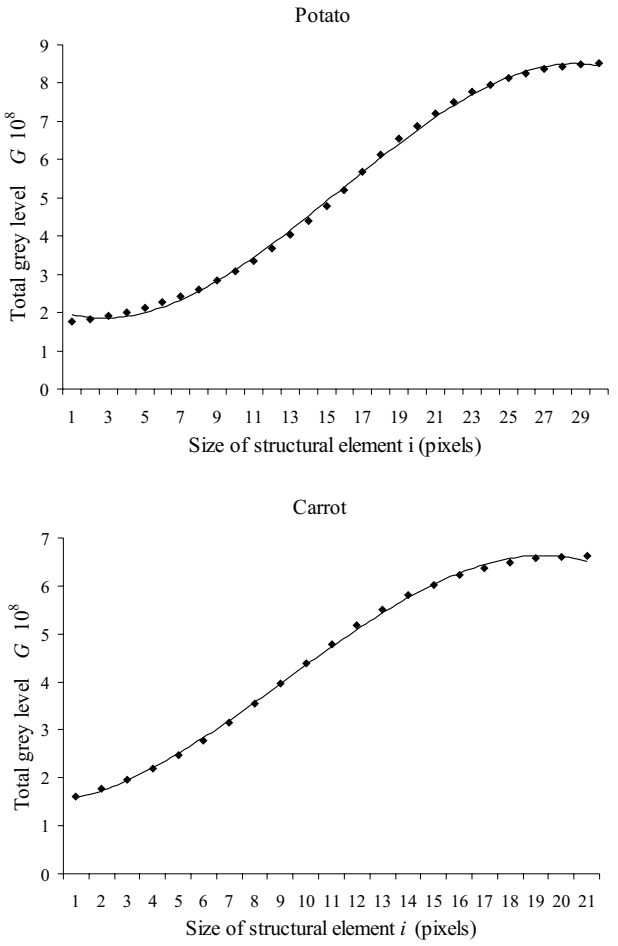


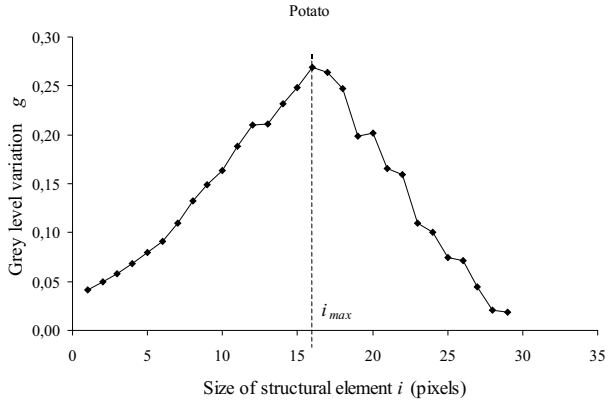
Fig. 3. Change of the total grey level G with increasing size of the structural element i.

the third-order polynomial curve has the inflection point. Thus, the size of the structural element at the inflection point is equal to $-b/3c$, where b and c are coefficients in equation:

$$G(i) = d + ai + bi^2 + ci^3, \tag{2}$$

where: i is the size of the structural element.

Since the size of the structural element i has discrete values, the maximum of the secant modulus *ie* maximal gradient of $G(i)$ function (Eq. (2)) can be obtained also by analysis of the $g(i)$ function (Eq. (1)). In Fig. 4, the g value is presented in a function of the size of the structural element i. Comparison of the size of the structural element at the maximum of $g(i)$ function with $-b/3c$ values gives linear relationship $y=0.96x$ with the correlation coefficient $R^2=0.68$ for potato and linear relationship $y=1.03x$ with the correlation coefficient $R^2=0.76$ for carrot. The deviation from the ideal linear relationships is result of the estimation of discrete values i_{max} from the graphics similar to Fig. 4.



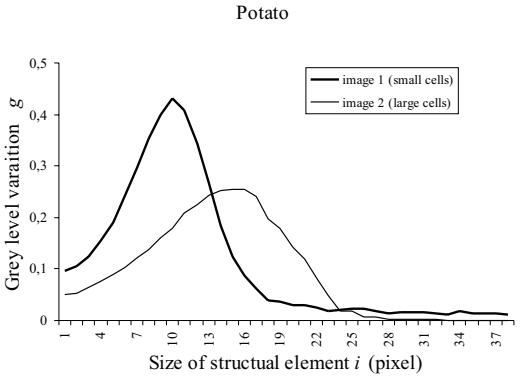


Fig. 5. An example of grey level variation $g(i)$ function for two images obtained from different potatoes.

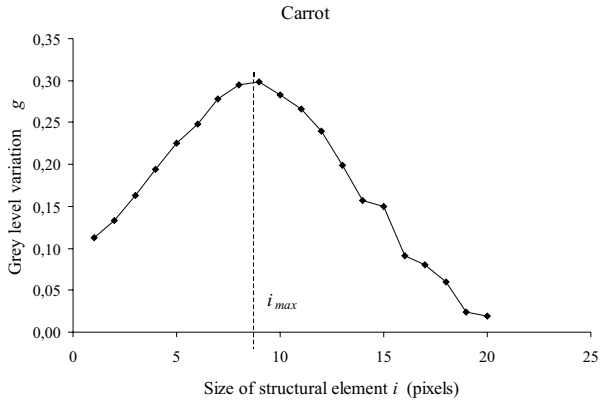


Fig. 4. Changes of grey level variation g value in a function of the size of the structural element i of potato and carrot. The i_{max} is the size of the structural element at the maximum of the g value.

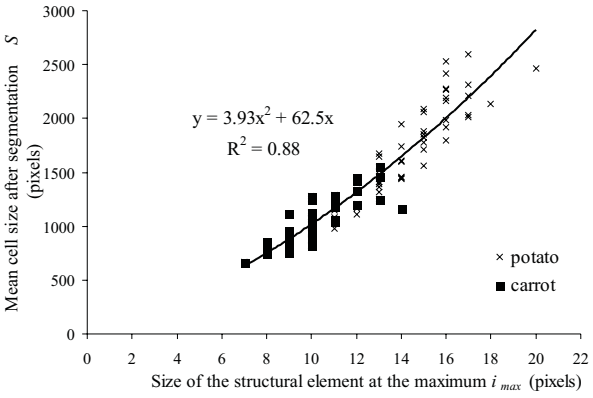


Fig. 6. Comparison of the size of the structural element i_{max} at the maximum of the grey level variation with the mean cell size S obtained by image segmentation of potato and carrot.

The size of the structural element of closing operator applied to the confocal images can be used for estimation of the mean cell size within the image. In Fig. 5, an example of differences between $g(i)$ is shown for two different images of potato tissue. The distributions of $g(i)$ are different for these two images similar as it was when potato and carrot images are compared. In order to find a relationship between the size of the structural element of closing operator at the maximum $g(i)$ obtained by analysis of its distributions the two methods: segmentation and VTA were applied to the same 50 images of potato and 30 images of carrot tissue. Results are shown in Fig. 6 for potato and carrot. The relationships are second order polynomial:

$$S = ki_{\max}{}^2 + mi_{\max}, \tag{3}$$

where: S is the mean cell size obtained by the segmentation method, i_{max} – the size of the structural element at the maximum of $g(i)$, k , m are coefficients.

The Eq. (3) allows recalculating results obtained by VTA to real cell sizes of the cells from confocal images. The k coefficient for potato is equal 4.5 and for carrot 1.2, while m coefficient has value 53 and 91, respectively. The correlation coefficients R^2 are equal to 0.82 and 0.69, for potato and carrot respectively. However, plotting results for potato and carrot together shows that one recalculating curve would be used with higher correlation coefficient equal R^2=0.88, which is satisfactory for quantitative estimation of the mean cell size using VTA method (Fig. 6). The reason is that the confocal images for both materials were obtained using the same sample preparation and the same settings of the CSLM, thus the important features of the images for grey level variation after application of closing operator are also the same. The only one difference is in the cell sizes in potato and carrot tissues.

CONCLUSIONS

In the paper, the method for quantitative analysis of cellular skeleton of potato and carrot tissue has been developed. The most important features can be summarized as:

1. Visual texture analysis allows obtaining quantitative information about the size of the cells within images obtained by the confocal laser scanning microscope.

2. The quantitative information about the mean cell size within the confocal image can be obtained by both calculating inflection point on $G(i)$ curve or by grey level variation $g(i)$ analysis.

3. Common recalculation equation for obtaining the mean cell size can be used for both materials potato and carrot.

Taking into consideration the fact that visual texture analysis allows obtaining quantitative information about the cell size and eventually a thickness of the cell walls (this was not done in this experiment, but using opening operator is useful for this purpose) without segmentation the method would be used for analysis more complicated images. Next, the VTA method will by applied to very difficult images from a conventional optical microscope. For this purpose, a special sample preparation procedure and the same lighting conditions should be found out. The results of the present experiment have shown that the VTA method is promising.

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