DIGITAL ANALYSIS AND POTATO TISSUE IMAGE PROCESSING AT THE APPLICATION OF VORONOI'S DIAGRAMS*

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A b s t r a c t. In this paper image processing is presented from the point of view of obtaining cell wall image. We also proposed some measurement and analysis methods. Because of non-continuos character of plant structure, the authors applied Voronoi's diagrams. This model allows for application of some point co-ordinates and segment lengths only. Also Voronoi's diagrams make easier obtaining a few parameters important for geometrical properties of cell wall.

Color microscope images have been converted from RGB system into HLS system which enabled to obtain information about the space configuration of point of objects being investigated and to identify structural elements.

K e y w o r d s: image analysis, potato tissue, Voronoi's diagram, Fourier transformant.

INTRODUCTION

In the research on plant tissue and soil structure, an image itself is the basic element of gaining information, that is essential for an examiner, i.e., cell size, their density in the area unit, distribution in the area image etc. By the application of microscopic image encoded in the computer memory we obtain the results required without traditional measurement techniques.

Besides the above mentioned quantities the researcher would like to know how does it look, and what information has been carried by non planar specimen structure like unprepared plant tissue or soil. So the 3D non planar structure reconstruction method, required equipment for this aim, and some example results have been presented.

There are many techniques based on bit image structure, which are helpful here, e.g., counting pixels number per each image component or examining the colour saturation. It is worth mentioning, that the image recorded in computer memory is represented as a matrix the components of which are the values of individual pixels [2,7]. That is the reason why before starting the evaluation of parameters we are interested in, an image must be prepared in such a way, as if the values in this matrix were only binary. Then, we may evaluate what we are concerned with. Only a few ways of image processing, that help in extracting cell walls, are discussed here. We will approach the idea of Voronoi's diagrams. They are very helpful in describing cell walls from the point of view of image structure analysis of the potato tissue.

TISSUE IMAGE PROCESSING

Image analysis processing, by means of Voronoi's polygons can be divided into 2 stages:
1) binarization of the input image,

2) vectorization of the binarized image.

In the first stage it is important to process the input image so as it would be only black and white (without greylevels). It will allow for defining what an edge is. The second stage deals with the processing of the above mentioned matrix that represents an image into data sets of vertices and edges, in other words, the processing from the bit into vector image of binarized image. Image vectorisation allows for point co-ordinates and segments lengths.

Figure 1 presents the image grabbed by the CAMA 10 camera from confocal microscope COCFOCAL 2002 [3,4,6] into computer memory. The possibilities of this camera enable to grab the image of high contrast. The camera specificity enables the observation of very thin slices of a specimen. So if we set the microscope so as to show only the plane where cell walls tissue edges are, we will observe only cell walls perpendicular to the specimen surface. It does not exclude differently placed cell walls on the same plane. The specimen is observed a few minutes after cutting a potato. The cutting is made in water. This makes air flow into the cut cells impossible. Moreover, their contents has been rinsed out. Only the cell walls remain unchanged. After grabbing image into memory the image

becomes the matrix of integer counts of the following size: 700 x 550 pixels. Every greylevel is represented by one number out of 0-255 range. On the computer screen, the content of this matrix is visible as an image. In the image cells walls, the edges are the brightest ones.

In the right bottom corner, the edges are not so much visible. It results from the fact that they do not belong to the plane being observed. Apart from the edges, also some cell walls are visible. So the inside part of the areas limited by the edges should be removed not to cause big mistakes during processing. Next, using filters we eliminate noises from the image.

If we maximally brighten up the image, it will turn out that where eyes do not notice anything, for the computer there is still some value, for instance 4, 10 etc. Human eyes do not react to such greylevels and give false information about the image. To extract these greylevels we need specific filters, which will process an image so as to highlight the pixels (increase matrix value), which are not visible. It is more necessary for an analyser than for an eye so as the pixels representing edges were properly separated from the background, i. e., that what is not the subject of the analysis. As we see in the input image in the right bottom corner, the eyes notice nothing and in a binarized image we can see these edges very distinctly.

The cell walls thickness, as the subject of the analysis, is troublesome. In the input image it is difficult to distinguish that what is already an edge and vice-versa. Our eye is able to distinguish the edges because it is taught by nature that an edge may appear there, where colour or greylevel changes. To cut it short, our brain causes the fact that we see darkly on the darker side and brightly on the brighter side. We use special filter for the computer to distinguish edges. This filter, on the border of two levels, increases the difference of their values and on the screen we can see distinct edges. But there is one problem whether that difference is the brightness jump, which corresponds to the real edge.

To solve this problem, a minimum value

Fig. 1. The image of cell walls edges of a potato.

is being arbitrarily planned. This value is interpreted as the background and that what is above the background is already the edge. This value can also be chosen as the image or some area of the process image average brightness. After choosing the edge, the operation, which binarizes the input image, is carried out (threshold) [7]. The second border, e.g., 0-128 is set up and the greylevels corresponding to higher or equal to 128 values are changed into 255 value (white colour), whereas the values below 128 are changed into 0 value.

If we have an adequately prepared image then all the values that we want to preserve after the processing should have value bigger than 128 value. That what is not the subject of the analysis should have the value lower than 128. After this binarization an image possesses some elements, which could not be removed by this operation - the so called artefacts. They are removed by the operations carried out on the binarized image. There can also be some distorted elements of the image subjected to the analysis, i.e., unclosed edges, area of not uniform in colour. These defects are removed by morphological operations on pixels, i.e., opening and closing the edges.

Now, after binarization, the image is subjected to skeletonization, i.e., skeleton estimation (in the mathematical sense) of the object received. Next, it is subjected to the operation of removing lines which are in contact with other lines but only from one end. It is called branches cutting. As a result, we get lines which are pixel thick and present walls. If after this operation there are some artefacts, then we can apply dilation to make the lines thicker. Thickening the lines allows for removing the artefacts found after skeletonization and branches cutting. After that, we can estimate and cut the branches again. This procedure can be repeated several times and then, we get the image presented in Fig. 2, but still this image is not Voronoi's diagram. Only estimation and connection of the vertices by the straight lines allows for creating Voronoi's polygon set. Having the vertices, we are able to estimate any diagram generators.
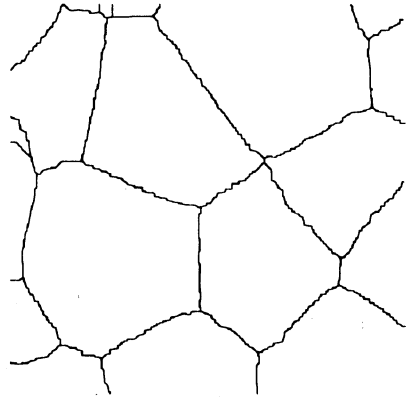

Fig. 2. Potato tissue image after skeletonization and branches cutting.

THREE DIMENTIONAL TSRLM IMAGES RECONSTRUCTION

There are two different kinds of confocal microscopes according to the light source used for the illumination:
- laser confocal microscopes,
- tandem scanning microscopes.

In the tandem scanning microscope the image obtained by the illumination using non-monochromatic light is a projection of the nontransparent three-dimensional surface onto the plane perpendicular to the optical axis using the chromatic aberration phenomenon. A light source in the TSRLM used in our experiments and presented in this report is the high intensity mercury lamp.

The effect of chromatic aberration causes that the image is coloured and the colours are related to the depth of the point being observed (the smaller wavelength the correspond the deeper point in the observation layer). The light source used has the spectrum from yellow to violet (Fig. 3).

On the basis of the properties above a topographic map of the surface being observed together with its representation in 3D space was made. Using a computer system of image acquisition and analysis the colour image was send from the microscope to the computer. The problem was that the image in the computer is represented using three RGB components. Utilising the property that any colour can be composed of any three noncomplementary basic colours and the fact that the resultant impression of hue is

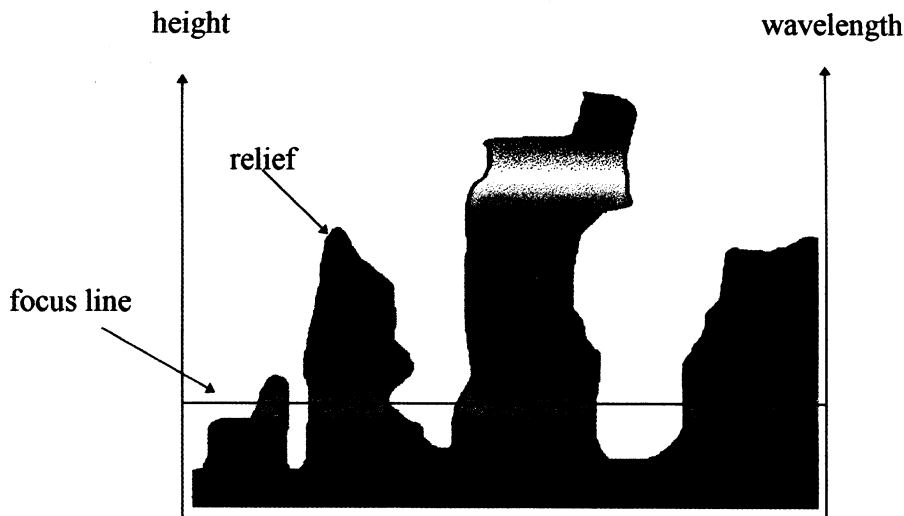height   wavelength

relief

focus line

Fig. 3. Principle of creation of a confocal image.

univocally prescribed to a value of length of electromagnetic wave we have converted the image from RGB system into image in HLS system (Fig. 4). The unique relation between the hue impression and the wavelength for a three chosen RGB filters is shown in Fig. 5.

In the HLS system, like in RGB system, the image is presented by three components:

a) hue (H) corresponds to the wavelength being observed or that of maximum amplitude;

b) luminosity (L) of the image in a given point is related to the total energy of light in this point;

c) saturation (S) is related to the spectrum of light. For a monochromatic wave saturation equals 100 % whereas it is equal 0 % for the gray shades in which all wavelengths from the visible range are present.

Utilising these properties of the HLS representation and the tables of conversion from wavelength to the depth (determined separately for every magnification of the microscope) using H component one can calculate the position of any given pixel of the image in 3D space. The next component (L) denoting the luminosity of a point can be used for the identification of the observed objects according to

the value of the coefficient of epiluminescence. The third component (S) after considerations of the spectrum characteristic effect of the light source and after correcting the filter characteristics (depth/wavelength) can give the information about mean gradient of the surface roughness inside one pixel.

The recognition of the objects based on the value of the reflection coefficient of light needs the angle of the incident light to be taken into consideration. Therefore the informations about the surface gradient of the image which can be obtained from H component (mean gradient for several pixels) as well as that obtained from S component (mean gradient for 1 pixel) are simultaneously required.

VORONOI'S DIAGRAMS DEFINITION

At first, we will present an intuitive definition rather than a mathematical one. To define the Voronoi's diagram (Fig. 6) we will consider the plane in which there is a set Z of freely distributed points. A number of these points is finite and bigger than two. Uncovering is the next assumption. Now, every position of any point in the plane will be connected with the nearest point from our set Z. If the distance from the point is the same as for two or more
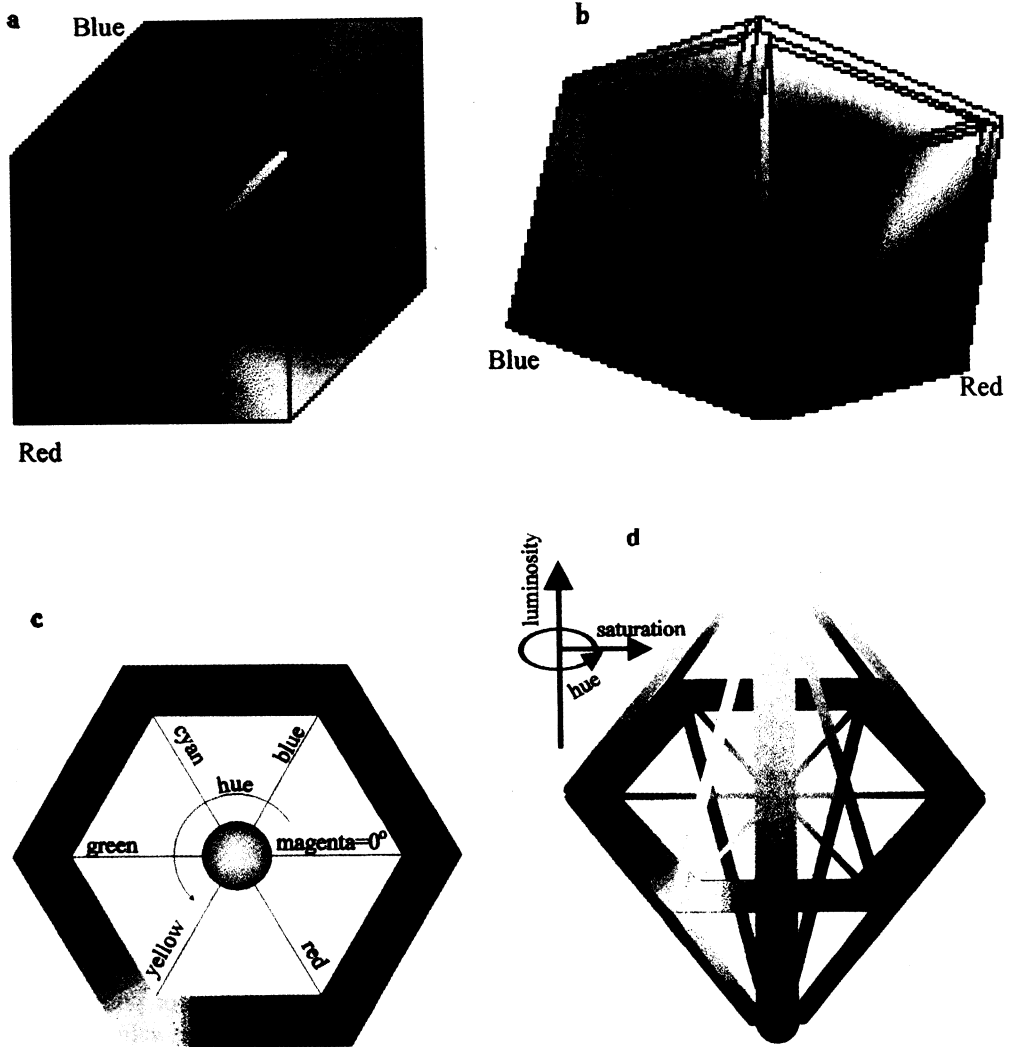
Fig. 4. Conversion from RGB image model to HLS image model: a) RGB model, b) RGB model after rotating in 3D RGB space, c) view from top on model B along balck-white axis, d) full HLS model.

points of the set Z then we will connect them with these two or more points. In this way, we can assign every plane point to at least one element from our set Z. All the points which are assigned to only one element form a half closed set around it. This set forms a polygon, whereas the points which are assigned to two elements, i.e., distance to both elements is the same, form the edges of these polygons. Points, which are assigned three or more elements, form vertices of these polygons. Plane division into such polygons is called Voronoi's diagrams whereas polygons - Voronoi's polygons. Full mathematical definition and description of Voronoi's diagrams is in [5].

In the current paper we are interesting in Voronoi's Diagrams for a few reasons. We using them to convert image to Voronoi's polygon generators and make easier counting of cells. Most frequently used cell counting algorithms are based on shape recognizing. They are rather time consuming operations. It seems to be easier
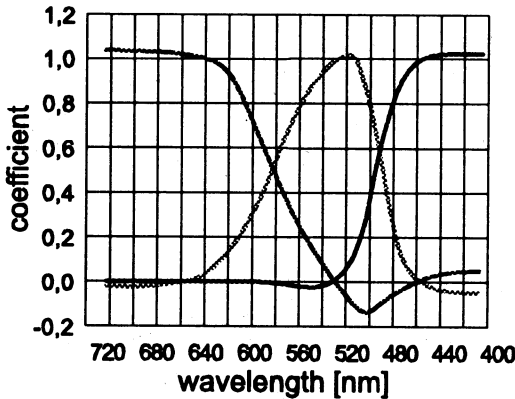
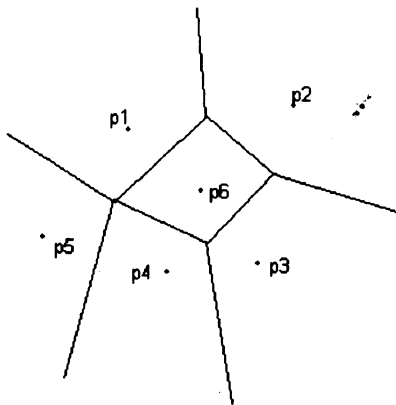Fig. 5. Composition of desired wavelength from three choosen filters RGB.

Fig. 6. Voronoi's diagram.

to count generators Next we need them to approach real potato tissue images and next to obtain cell wall direction distribution.

IMAGE ANALYSIS

After obtaining the data mentioned above, we can start the estimation. These data may be essential for both quantitative and qualitative estimation. Now we will consider only some pieces of information.

Area cell density

Area cell density is defined as cell number per area unit:

$$\xi_s = \frac{n_k}{s_j} \qquad (1)$$

where ξ_s - area cell density, n_k— number of cells, s_j - total cell area.

We do not have to find every cell. It is enough to count the number of generators in a certain area and we get the value required. Then, our formula is a little modified:

$$\xi_s = \frac{P_k}{s_j} \qquad (2)$$

where: P_k - stands for the number of generators of a certain area.

Examination of cell walls directions distribution

The best solution will be the application of Fourier's transformations [1] of these images. Simplicity and convenience of this analysis is based on the fact that the image is treated as a two-dimensional wave. Brightnesses are its amplitudes so we can estimate its spectrum. If we have only one-dimensional frequency distribution from one dimensional wave, then the spectrum of two-dimensional wave is like a two-dimensional image. As a result, we obtain an image again. An image for Fourier's analysis purpose can be defined as plane waves harmonic superposition $F(f_x, f_y)$ of frequency $f = \sqrt{f_x^2 + f_y^2}$ and direction of which angle tangent in relation to axis X $tg\alpha = \frac{f_x}{f_y}$. So transformant image is a set of points $p(f_x, f_y)$ whose co-ordinates determine value and direction of component wave, whereas its brightness is proportional to component wave amplitude. If there is a long and thin object (e.g., dash) in the image then, on the transformant image, we see that the component wave frequency of this element falls into lines perpendicular to the dash (Fig. 7). In the case of the structures similar to the potato tissue images being considered, we obtain transformants as on the Fig. 8. On the transformant, there are lines of dominant directions on the left side image (perpendicular to the given line). So by measuring angles on the transformant we obtain cell walls direction distribution in a potato tissue. If we take original image of potato tissue (Fig. 1) transformant will
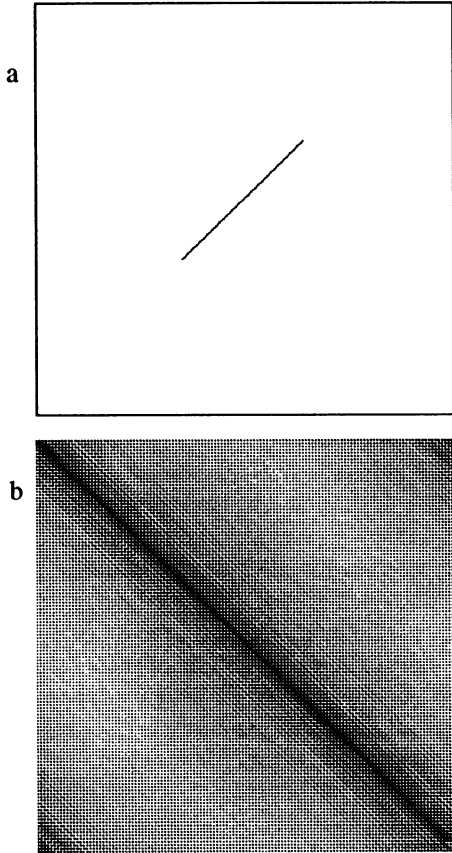
Fig. 7. Dash image and its transformant: a) original image, b) transform of image a.
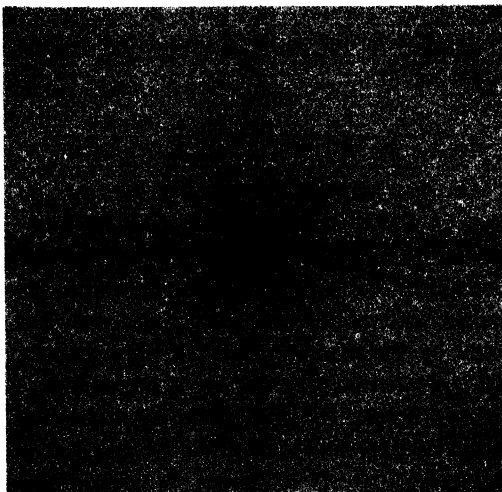

Fig. 8. Transform of image from Fig. 2.

be very fuzzy and analysing such image is very difficult but if we apply to FFT Voronoi's diagram obtained from previous image processing then our transform will be clearly and lines on it will be thinner. Therefore we can easily then from original image estimate cell wall direction distribution.

RESULTS

Table 1 shows the results of measurements for 10 images of sample potato intersection of the area 2 cm chosen at random. The application of this method for measurement, e.g., changes in structure because of stress occurrence, could be useful for obtaining the information about size deformation depending on stress. The data in the table are presented in degrees. While analysing them it is easy to notice that there is a correlation between the number of walls and the number of directions of a certain area (Fig. 9). It seems to be natural since the more walls and consequently cells in a given area the more possibilities of ordering walls. In the direction distribution of a certain area there are no directions chosen on purpose. Deeper discussion requires more measurements. Information which we get in these measurements is 2D and we can consider only perpendicular data for optical axis. We cannot get information about processes in 3D. Theoretically, if we were able to get a 3D image and transform it into Fourier's transformant then we could say something about real shapes
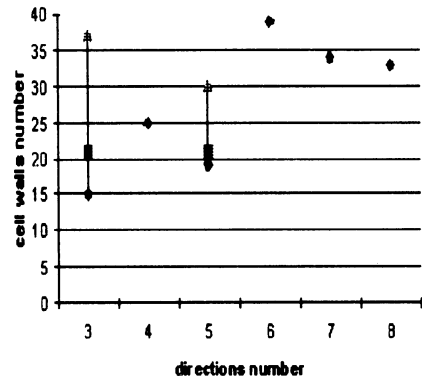

Fig. 9. Direction number versus cell wall number.

T a b l e 1. Exemplary measurements for 10 images of sample potato intersection

Image	Walls number	Directions toward OX
1	25	20.52 ± 0.08
		45.06 ± 0.12
		14.67 ± 0.48
		24.22 ± 0.26
2	30	112.98 ± 0.29
		134.47 ± 0.18
		143.91 ± 0.07
		17.21 ± 0.26
		34.91 ± 0.39
3	39	135.51 ± 0.32
		64.70 ± 0.24
		3.14 ± 0.18
		6.16 ± 0.21
		45.25 ± 0.19
		64.01 ± 0.22
4	37	54.12 ± 0.18
		42.37 ± 0.29
		63.19 ± 0.16
5	21	110.43 ± 0.27
		25.97 ± 0.07
		63.83 ± 0.39
		9.23 ± 0.26
		33.77 ± 0.11
6	15	8.36 ± 0.36
		3.98 ± 0.15
		7.88 ± 0.33
7	21	130.01 ± 0.16
		7.53 ± 0.41
		6.73 ± 0.52
8	19	103.77 ± 0.06
		132.99 ± 0.20
		148.01 ± 0.16
		47.66 ± 0.08
		70.97 ± 0.55
9	33	105.81 ± 0.34
		16.42 ± 0.31
		157.49 ± 0.30
		21.72 ± 0.33
		39.99 ± 0.63
		59.81 ± 0.12
		70.62 ± 0.30
		82.21 ± 0.34
10	34	97.20 ± 0.11
		11.52 ± 0.04
		40.53 ± 0.11
		7.20 ± 0.10
		35.79 ± 0.25
		48.23 ± 0.12
		9.16 ± 0.19

of cells and their changes. Now we can discuss 2D intersections of 3D cells, 2D projections of strain components.

The idea of three-dimensional reconstruction was used for the investigations of plant tissue samples. Reconstruction procedure has been performed for potato tissue image (Fig. 10).

Three-dimensional reconstruction was performed using H component only. Actually, software for full reconstruction of images with identification of structural components of objects using all three HLS components is under elaboration and improvement.

There is an uncertainty of determining the spatial position of image point for luminosity close to extreme value, because, in consideration of discrete RGB space, there are less RGB points in those regions in comparison to the ones where the luminosity is close to 50%. When the image points luminosity is close to 100 % it is possible to overcome the uncertainty either by making another image with lower illumination or by extrapolation of results from pixels of certain position in space. Points with luminosity L close to 0 % are either under illuminated or there is no light reflection in this point of observation layer. We can distinguish this two cases by making another image using higher illumination.

When the thickness of the investigated object is higher than that of the observed layer it is possible to perform 3D reconstruction using several subsequent images of the object. The resolution to depth is limited by the transparency of the object only. It enables 3D reconstruction similar to that in computer tomography as far as the investigated objects is transparent.

CONCLUSIONS

On one hand in the present research, an image is a very important carrier of information, but on the other, from the point of view of an examiner, the same image also comprises redundant information. Moreover, the data expected by the examiner are not very precise. That is the reason why our eye is not an objective 'instrument' for both gathering and interpreting the data contained in the image.

Image analysis turns out to possess devices for objective processing and analysis of the information contained in the image. In this
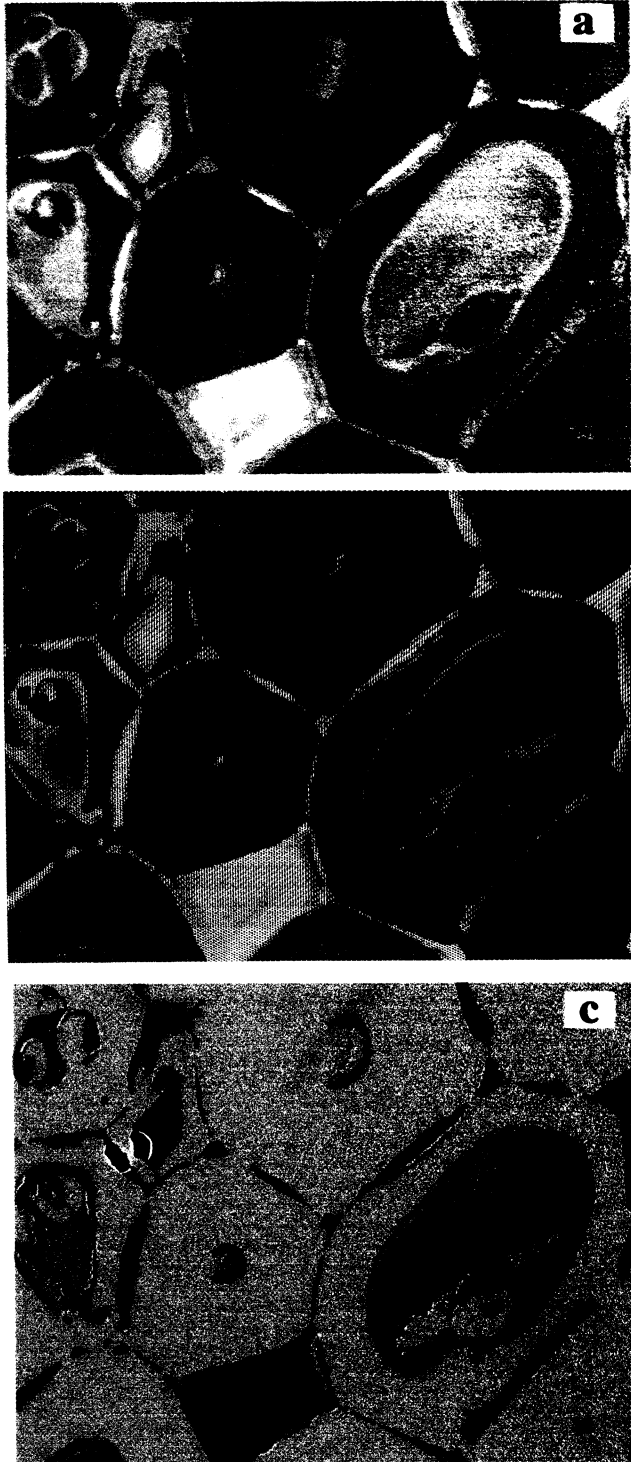
Fig. 10. Reconstruction of a 3D surface of plant of potato tissue: a) original image, b) topographic map of the sample surface in grey scale, and c) pseudovisualisation of a 3D surface.

paper image processing is presented from the point of view of obtaining cell wall image in a rather shortened way. We also proposed some information measurement and analysis methods. Because of non-continuous character of plant structure, the authors applied Voronoi's diagrams. This model allows for application of some point co-ordinates and segment lengths only.

It is an advantage for image analysis as far as the time of work on a computer is concerned. We also presented some example measurements obtained by image analysis. Here the information was provided by Fourier's transformant. Without statistical application we can observe the cell walls distribution in the photographed tissue.

Tandem scanning reflected light microscope together with computer image analyzer was used for a three-dimensional reconstruction of surface images of soil samples and plant tissues. Colour microscope images have been converted from RGB system into HLS system which enabled to obtain information about the space configuration of point of ob-

jects being investigated and to identify structural elements.

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