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The study of plant tissue by optical coherent microscopy method

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Abstract. The article presents the results of application of the optical coherent microscopy technique using a high-resolution automatic Linnik interference microscope to study the structure of plant tissues exemplified by surface periderm layers of a tuberous nightshade (*solánum tuberosum*) bulb. The results of 3D visualization of the structure of the sample under examination are provided. Scanning depth was 32 μm, with axial and lateral resolution of the device 1 μm.

1. Introduction

Research into surface and internal structure of various tissues are in demand in many areas of science and technique. To study biological objects, it would be of prime importance to save integrity of a sample under examination. The technique of optical coherent microscopy (OCM) offers the possibility to visualize not only an object surface, but also its internal structure, without disturbance of the same.

2. Method and appliance

The OCM applied possesses high axial and lateral resolutions at the depth of optical radiation penetration, which often surpass characteristics of similar optical and other methods of examination. It works according to the principle of measurement of the extent to which optical radiation reflects from the object examined at different depths, based on light interferometry. In the course of measurements, an object is lit with optical radiation, which, while penetrating the medium reflects from layers of the sample varying in depth to different extent. Determination of reflectivity for each point of certain layers of the medium examined permits to get information on internal structure of an object of examination, and to restore its three-dimensional structure. In this paper, the samples examined were illuminated with visible range beam, with a halogen lamp used as a source of radiation. A two-beam interferometer is a key element of OCM system. In this research, a high resolution automatic Linnik interference microscope was used running in two modes, combining advantages of a traditional two-beam interferometer and of a high-magnification optical microscope (up to 500^{x}). The area of the observation surface was 200x160 μm. Therefore, it allows for examination of both an interference pattern and surface of the object examined [1]. Lateral and axial resolution of the device was 1 μm.

3. Object of study

This research considered near-surface layers of cover tissue of a bulb of tuberous nightshade (*solánum tuberosum*), i.e. periderm. Periderm of the object investigated represents a multilayer structure built of tissue of different types and structures, at different depths from surface. Considering the functional purpose of periderm, OCM technique may reveal and measure its structural elements. In the course of

work, different sections of several samples were examined, namely, those with no visible defects, those with mechanical damage, and areas with swellings and other structural abnormities. The tomograms revealed differences between structure of defective tissues and healthy periderm layers [2].

4. Research and analysis of the visualization results

At the initial stage of research, plant samples were examined under microscope by transmitted light, using a bright-field method to detect the areas of interest. The samples selected for further research were scanned with variable parameters to choose scanning criteria allowing for the best visualization under current conditions. The rated values provided scanning depth 32 μm. Applying the OCM technique and software processing of video image data array resulted in construction of tomograms of the areas researched, which can be interpreted as images of cross sections of the samples scanned. Tomograms of surface periderm layer are discussed in [2]. Visualization of internal structure allows not only for a conclusion of an abnormity of tissues without disturbing thereof, but also for further observation over progress of a disease as exemplified by the same specific area.

Further software processing of experimental data was followed by three-dimensional pictures of the areas scanned, one of which is represented in Fig, 1.

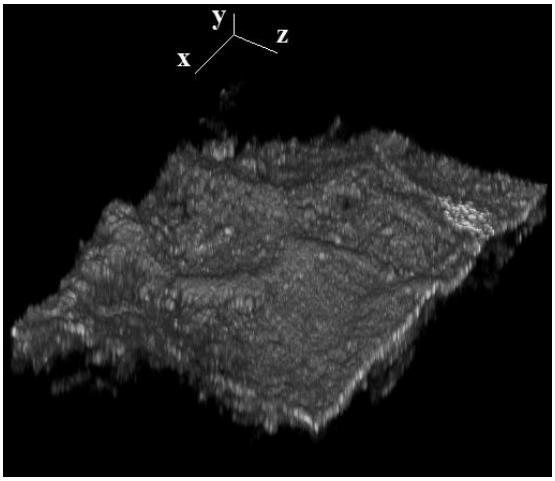


Figure 1. Reconstructed three-dimensional model in the scanned area

The 3D-patterns constructed permitted not only to view surface of the samples from different perspectives, but also to get tomograms of the objects in any section of interest. Therefore, 3D images made it possible to detect and identify certain structural elements of periderm of the samples visualized. Besides, three-dimensional visualization results offer the possibility to detect and measure structural elements, both observable in pictures of surface, and inaccessible for traditional microscopy.

Thus, the initial stage of the research involved a healthy area of periderm. The still picture of surface obtained through a high resolution automatic Linnik interference microscope showed no defects or structural elements. However, upon viewing the three-dimensional pattern of the area concerned from the same perspective, a so called lenticel in the sample periderm was observed. This is a structural element participating in a metabolic process between the bulb and environment.

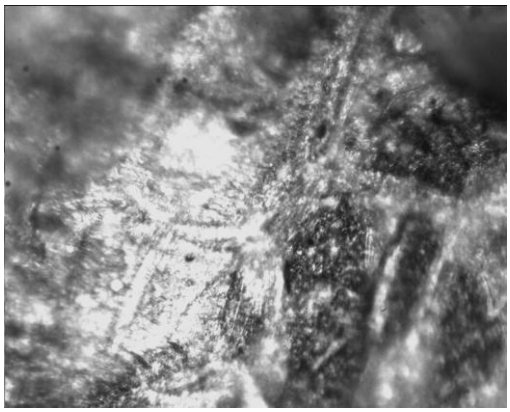
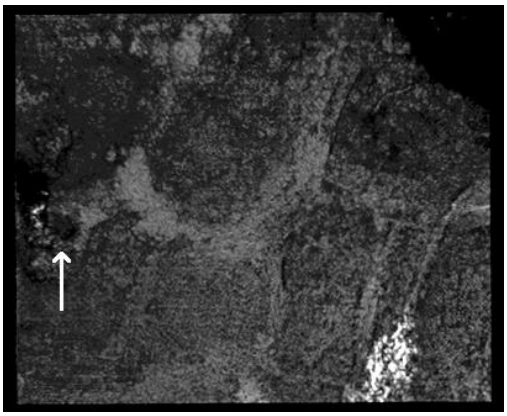
(a)  (b) 

Figure 2. Image of the surface observed in the microspore mode (a), image of a horizontal profile of a three-dimensional digital model (b)

Detection of the lenticel determined more detailed study of the area concerned, construction of tomogram in specific cross-sections, and further measurement of geometric parameters of the structural element detected. Based on the tomograms of the lenticel shown in Fig. 2 in different sections, the diameter of the lenticel was measured (4 μm).

These measurements let us conclude that the sample examined is healthy, since according to biological research [3], diameter of a lenticel of a healthy bulb of tuberous nightshade ranges from 3 to 8 μm depending on the grade, which complies with the measurements. Control over diameter of lenticels in the bulb provides diagnostics of viral and other diseases of tuberous nightshade. Thus, using a noninvasive optical coherent microscopy technique, we can watch changing in a state of lenticels of the same bulb, without interference with the process of disease. Therefore, the said technique is applicable not only for study of structure of plants, but also for research into different abnormities thereof.

5. Conclusions

The combination of two high-resolution digital optical methods for diagnostics of diseases and assessment sizes of periderm surface defect may be useful for investigations of other plants' periderm. The study of periderm by the high-resolving full-field optical coherence microscopy method showed that in order to observe the microstructure of layers located deeper from surface, it is necessary to use a more powerful source with emission in the near IR spectrum area and greater scanning depth. Also, in order to enhance the conditions of plant texture visualization, it is necessary to carry out additional studies of spectral characteristics of individual tissue textures of the object. We believe that the results of the study can be helpful to a wide range of specialists.

6. References

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