The study of plant tissue by digital microscopy methods

V V Chirskaya¹, N B Margaryants¹, E V Zhukova¹
¹Saint Petersburg National Research University of Information Technologies, Mechanics and Optics, 49 Kronverkskiy pr., Saint Petersburg 197101, Russia

E-mail: fosp@grv.ifmo.ru

Abstract. Results of the study of potato tuber (Solanum tuberosum L.) periderm with the use of digital microscopy methods are considered. A light microscope was used for diagnostics of periderm surface and recording of the images of studied sections. High-resolving full-field optical coherence microscopy ensured obtaining tomograms images of periderm texture in depth. The scanning depth was 32 µm. Tomograms (B-scans) of surfaces with normal structure of periderm upper layer in the area of mechanic damage of external protective tissues and also in the area of growth caused by a fungal disease are presented. Tomograms resolution by depth and in lateral directions is 1 µm.

1. Introduction

Optical coherence microscopy is a modern method of investigation the microstructure of various materials and substances. When studying living biological tissues, the employment of this method ensures the nondestructive nature of experimental work and creates conditions for visualization of tissue texture with the resolution that is required for diagnostics. Traditionally, this method is used to study the texture of biological tissues in order to advance providing diagnostic medical assistance and scientific study of biological processes of development and degradation of cellular and tissue structures. Plant tissues are also widely studied using optical coherence microscopy. For instance, the results of tradescantia leaves cells visualization are discussed in [1] and research on the influence of viral infections on the change of orchid tissue is described in [2]. The progress of studies conducted with the help of optical coherence microscopy methods depends on device resolution, software speed and effectiveness of computation algorithms when working with large data volumes, and, also, on the method of presenting information obtained for tissue texture on micro level. This article deals with the results of study of the potato tuber (Solanum tuberosum L.) periderm. The purpose of the work was to make a comparative study of periderm tissue for various types of its surface damages.

2. High-resolving full-field optical coherence microscope

The experiment was performed using a high-resolving full-field optical coherence microscope, whose structure combines the advantages of a low-coherence Michelson interferometer with Linnik configuration and the light microscope [3]. A halogen lamp with capacity of 70 W served as a broadband light source. A beam-splitting plate formed two parallel coherent beams, which were focused respectively on the object surface and reference mirror with the help of two micro objectives that have similar characteristics. Radiation reflected from the object and the mirror was collected by micro objectives and two coherent beams again went through the beam divider. Further, with the help of the lens, the beams were brought together on a plane of video-camera matrix, which is used for the registration of the observing interference pattern. The optical compensator was placed in the channel of reference beam, which allowed to bring in additional path difference for improving the contrast of
interference patterns. The microscope magnification was 500X and the area of the observation surface was 200x200 \( \mu m^2 \).

In experiment the stepper motor through fixed interval (6.4 nm) shifted the micro objective by depth with regard to the surface of the object under study. The optical path of the reference beam always remained the same. Owing to small shifts of the micro objective and small sizes of optical inhomogeneities, the conditions for observing the interference effect were met. At the specified scanning depth, an optical path difference between subject and reference beams for a number of waves was less than the coherence length of this type of source. A total interference pattern was observed at the focal plane of a lens. When changing the scanning depth, the conditions of interference observation were carried out for another waves.

Recording of video frames with the images of interference patterns was carried out jointly with the changing of the scanning depth through the equal intervals (64 nm). During the experiment, a depth of scanning was limited by 32 µm. USB 2.0 digital video-camera Color VEC-135 (1.3 MP) was used to record interference patterns, and registration of the interferometric images was carried out in the visible area of the spectrum. The size of the recording image was 1280x500 pixels. Combined computer processing of the array of the interference images (500 video-frames) with the help of a specially developed algorithm allowed to obtain the images of plant tissue tomograms (B-scans) for selected sections within the area of object surface observed with the microscope.

As a result of research, the tomograms of potato tuber periderm were obtained. The size of B-scans was 32 µm in height (scanning depth) and 200 µm in width (scanning length). The resolution of tomogram image by depth and in lateral directions was not more than 1 µm. Such scanning parameters allowed to examine the upper layers of potato tuber periderm.

3. Results

The tomogram of the periderm surface without defects is presented in Figure 1. The image provides normal layered tissue texture. For comparison, Figure 2 presents the tomogram obtained in the growth area, formed on periderm surface during potato tuber growth under the influence of plant pathogenic fungi. Such growths are formed in case of tissue infection with the fungi of _common potato scab_. The tomogram shows the profile of growth in the selected section. It is seen that the periderm tissue loses layered cellular texture.

![Figure 1. Tomogram of potato periderm without defects](image)

The height of the growth at the section under study was about 17 µm, the length of its base was within the limits of 149 µm. Such estimation can be helpful for understanding the nature and possible tendencies in the defect development allowing the specialists to diagnose the character and the speed of tuber disease.
The tomogram of periderm section surface with mechanic damage is presented in figure 3. It is seen that the rupture in the external protective tissue also causes a local breaking of the layered tissue texture. Also, it can be seen that the area of displacement of external tissue layer corresponds to the local breaking of its structural elements. The surface relief form points to the absence of dangerous changes in the preiderm texture.

4. 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.

Acknowledgments
This work was financially supported by Government of Russian Federation, Grant 074-U01.

References
[1] Sapozhnikova V V, Kamenskii V A and Kuranov R V 2003 Rus. Plant Physiol. 50 282
[2] Chow T H, Tan K M, Hg B K, Razul S G, Tay C F, Chia T F and Poh W T 2009 J. Biomed. Opt 14 014006
[3] Alarousu E, Gurov I, Kalinina N, Karpets A, Margariants N, Myllylä R, Prykäri T and Vorobeva E 2008 Proc. SPIE of Advanced Laser Technologies (Finland, Levi, 03 September 2007) 702212