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CONFOCAL MICROSCOPY FOR INVESTIGATIONS OF AGRICULTURAL MATERIALS*

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A b s t r a c t. The paper presents the examples of various applications of the microscopic system made in the Institute of Agrophysics (IA) in Lublin, Poland and consisting of a Tandem Scanning Reflected Light Microscope TSRLM connected to a computer data collecting and image analysis system, containing a high-resolution and sensitivity CCD-HDD camera and a hardware system for data collection and image transmission. The computer image analysis system with its original software worked out together with TSRLM makes a new unit - TSRLM-IA.

The optical noise was eliminated as a result of a modification of the camera and especially written software, thus omitting the main disadvantage of the Minsky's Microscope, i.e., low intensity of the light reaching the ocular. The results presented were obtained during examination our samples of agricultural origin (potato tuber tissue, soil) with very different coefficient of the epiluminescence.

K e y w o r d s: confocal microscopy, agricultural materials, microstructure

INTRODUCTION

The Petran and Hadrawsky Tandem Scanning Reflected Light Microscope (TSRLM) has many advantages (high contrast, work in real time, simple construction, *etc.* [6]) making it possible to use it for analyzing samples of materials of agricultural origin [4]. The possibility to obtain images from the inside of semi-transparent objects predisposes it to investigate the inner structure of plant and animal tissues. In contrast to the Laser Confocal Scanning Microscope (CLSM), the images in TSRLM-IA are obtained in the time of single millisecond to a few seconds. It allows for monitoring the processes in real time or use long exposition time in order to eliminate optical and electronic noises during the observation in case of high magnification with water immersion.

The principle of operation of the laser microscope (CLSM) lies in the fact that light beam from the source illuminates point by point the selected plane in the object, and reflected light in aperture acceptable by the objective is registered in the detector. Then, in identical way, successive image plane is scanned, and so on. From gathered data the object image is reconstructed. Duration times of data collection and image reconstruction in the present design of the CLSM microscopes are two orders longer than in the TSRLM-IA microscope, while the contrast of obtained images is considerably better in the CLSM microscope (beam

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of the confocal laser light). The analysis of the parameters described above shows that both designs are complementary to each other in various applications.

RESULTS

Fracture propagation in potato tissue

In our studies concerned with the investigation of fracture propagation in plant materials, we have attempted to analyze the data obtained in the following experiment. We have made a number of bars cut with a roto-slicer DTY-7700 from potato tuber with the dimensions 5x7x35 (mm). The images of fractures obtained have been photographed in a confocal microscope Confocal 2002 using the CCD-HDD camera, and then analyzed using our image analyzing computer system.

Figure 1 presents a three-dimensional reconstruction of an example fracture. In order to obtain the image a number of scanning (twelve scanning) in the axis 'z' of the fracture obtained with a 'step' every 5 μm have been presented. The fracture obtained has not been prepared in any manner. Then, a conversion of the images obtained has been made in such a way that the 'z' axis is represented as the change of colour in the HLS system, and the scale obtained is presented beside the drawing [5]. As it appears from the 3D reconstruction presented, in this case the propagation of the fracture proceeded from tearing away undamaged cells (yellow part of the drawing) to tearing away 3-3.5 layers. The fractures obtained have not been additionally prepared, which allowed for the observation of their structure in an intact form (visible starch grains) at the places, where the fractured cells have been torn apart. This fact confirms our earlier assumption (Haman and Pukos - private information) that the destruction of a potato tissue starts with the 'unstucking' of cells.

Figure 2 (a-e) presents the images of subsequent layers of potato tuber tissue. The images have been obtained in TSRLM-IA microscope changing the distance between the preparation and objective with the 'step' every 5 μm. To obtain such images using a classical optical or electron microscope would require making and fixing a series of 5 μm preparations. The current techniques used for drying (drying with solvents, sublimation drying, and drying at a

Fig. 1. Fracture in potato tuber tissue with the scale in RGB system.

Fig. 2. (a-e).Subsequent layers of potato tuber tissue.

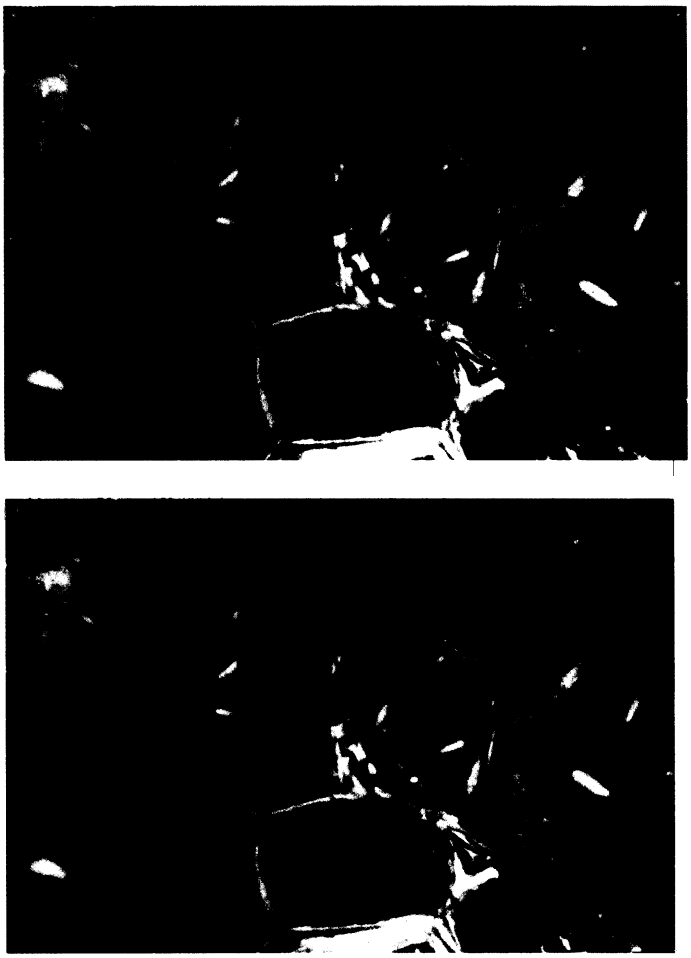

Fig. 2. Continuation.

critical point) change the geometric dependencies of the object being dried, and resin fixing in order to give the preparation appropriate mechanical properties for cutting destroys some of its elements. The object presented in Fig. 2 (a-e) has not been subjected to initial preparation and the images obtained maintained geometrical dependencies of an intact structure.

TSRLM-IA applications for the soil cros-section observation (Luminescence effects)

The tandem scanning confocal microscope is practically fit for scanning record of epiluminescence of objects being investigated.

In the TSRLM-IA, the light source is a mercury lamp, which makes it possible to induce luminescence of some substances used in microscopic preparation. This property can also be used for the observation of samples saturated with luminescent dies or those containing own luminophores.

In order to test the usefulness of TSRLM-IA microscope for the observation of soil samples in a luminescent light the following preparations have been made:

Two portions of epoxyacrylic resin monomer have been prepared and subsequently bromothymolic and methylene blue have been added. Then 40 % of PAC hardener have been

Fig. 3. Soil cros-section. Sample of the loess soil have been prepared with bromothymolic blue.

added. Loess soil samples have been the flooded with the solutions obtained in low pressure conditions. After the samples have been polymerized in a drier 45 °C, polished surfaces have been made. The polished surfaces have been polished using diamond paste with 2-5 μm grains.

Figure 3 presents the images of polished surfaces obtained.

Other applications

Transformation of tomograph image

The studies on mechanisms of soil compaction carried out according to traditional methods are very laborious. Soil samples, subjected to different loading, should be preserved by impregnation with resins. This process, however, is of low repeatability, because to preserve internal structure of deformed samples, the gaseous phase from the sample must be replaced by polimer of distinctly higher viscosity in low pressure conditions which, in turn, often causes the destruction of already fixed internal structure. Then, during cutting a sample into thin slices, part of material is destroyed. This does not allow to reconstruct the tested sample. Therefore, computer tomography has lately been used to study the internal structure of soil samples [1].

Our group, in co-operation with the Institute of Land Technics in Müncheberg (Germany), carried out investigations of soil compaction using the computer tomograph (Siemens).

Interpretation of obtained data from successive scanning (in photographic films) is very time consuming. That is why an attempt to process these data with the use of our system of data acquisition and image analysis in TSRLM-IA microscope, was undertaken.

Figure 4 (a-c) presents some of the computer tomograph data. The loess soil image of 3 following scans with the step of 1 mm were processed on the TIFF format images. Then they were conversed to HLS system [3]. Figure 5 presents a 3D pseudoreconstructions. Figure 6 shows the same image where axis 'z' is presented in RGB colour system.

Fig. 4 (a-c). Computer Tomograph images, 3 following scans with step of 1 mm.

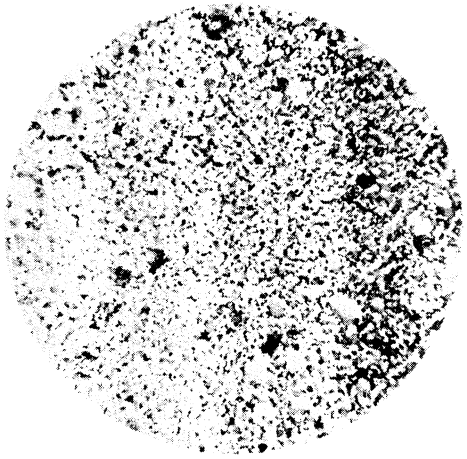

Fig. 5. Pseudo 3D reconstruction of 3 previous images.

Fig. 6. Transformation of the Fig. 5 where axis 'z' is presented in RGB colour scale.

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