



**FACULTY OF AGRICULTURAL SCIENCES & ALLIED INDUSTRIES**

**TECHNIQUES IN PLANT PROTECTION MSH-304**

## LECTURE 03

### **Introduction**

The lecture focuses on basics of electron microscopy. We explain that electrons can be treated both as subatomic particles and as waves. Next, structure of microscopes is shown and briefly described. As the third thing, we discriminate between transmission and scanning microscope, describe techniques used to prepare samples. As a last point – application of both types of microscopes in various branches of science is shown. We expect, that after this lecture pupils will describe electron microscopes, characterize their structure and mechanism of obtaining images. They will also be able to give numerous examples of electron microscopy usage in various branches of science, not only in biology, but also in medical or technical sciences. The whole lecture takes about 2 hours. It is illustrated with electronograms and finally evolves into discussion about possible usage of the electron microscopes nowadays and in a future.

### **Transmission electron microscopy –**

They are acquainted with this type of microscope in practice. The structure of TEM is shown, as well as ultramicrotomes, grids, fixatives etc. They can observe some of the steps of sample preparation, e.g. fixation, dehydration, embedding, sectioning. The final step of this part is observation of various biological samples under TEM. We emphasize high resolution and magnification power as two very important virtues of TEM. Pupils are taught how to distinguish between plant and animal cell, how to recognize various organelles on the basis of the number of biological membranes and other characteristic features. They try to find connection between number and location of specific organelles and their role.

We show for example gut columnar cells and pupils try to find connection between:

a) presence of microvilli in midgut cells and their role in these cells,

- b) high number and location of mitochondria and transport across membrane or presence of cilia,
- c) presence of rough endoplasmic reticulum and activity of gut cells.

Due to complicated and time-consuming method of sample preparation, we do not organize practical course concerning TEM.

### **Scanning electron microscope –**

As in case of TEM, they are familiarized with the microscope, additional equipment (sputter coater, critical point dryer) and methods of sample preparation. They observe the simple method of preparation of dry and hard samples like hair, seeds, arthropods, coins etc. After that, they can observe these samples under SEM. By showing pictures of the same objects taken under light microscopes, we put special attention to the resolution as advantage of SEM. Participants of the course are also acquainted with software which enables measurement of various structures. For example, the participants give samples of their hair. Then they can compare their condition and refer it to cosmetics they use (or they do not use), measure the thickness of hair. Here, we put special attention to circumstances, which are necessary for properly carried out experiments (control, measurement at the same height of the hair, repetition). After that, we smoothly move to the next point – participants plan to organize their own research using scanning microscope.

### **Plan of the experiments on SEM**

First, they make a kind of “brainstorming” within their groups. Then, they share their ideas (they state a research question, a hypothesis, show the plan of their experiments) with other groups. After constructive discussion, they can modify/perform their experiment or if they are unlucky, form reorganize their research plan. Under supervision of university teaching staff they plan the schedule of the experiment. After some hours, in the next day, they bring their samples and prepare specimens for SEM.

**Scanning electron microscopy - practicals** After planning, pupils carry out their experiments. Here are some examples of research questions:

- a) What material is the best for bike saddle production?

- b) Do different types of chocolate differ in structure?
- c) Effect of eyelash cosmetics on their thickness.
- d) What is the size of pollen grains of various species?
- e) Structure of eggshell of various species