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SAMPLE PREPARATION FOR TRANSLUCENT AND SCANNING ELECTRON MICROSCOPY: NEW *LEICA MICROSYSTEMS* COATERS

The research deals with new Leica Microsystems desktop coaters used for application of conductive layers in vacuum. Their technical specifications and scope of application have been described.

Key words: nanotechnology, electron microscopy, microstructure analysis, replica, automatic control, and vacuum level.

The electron microscopy is a method for studying the structures which are beyond the visibility limits of light microscope and have dimensions less than one micron (1 micron to 1-5 A). There are two main directions of electron microscopy: *the transmission* (TEM) and *the scanning* (SEM) ones, both based on the use of appropriate types of electron microscopes and giving qualitatively different information about the subject of research. Often, both types of electron microscopes are used together.

The most difficult part of electron microscopic studies is the sample preparation, since the electron microscope is a precision instrument that requires special methods of making preparations to identify the individual components of subject of research (cells, bacteria, viruses, etc.), as well as to preserve their structure under the action of electron beam in high vacuum. When preparing the samples both *direct* and *indirect methods* are used.

The direct method is a method of ultra-thin specimen. The ultra-thin specimens are prepared in various ways: by cutting on ultramicrotome; by etching or electro-polishing; by melting with casting of thin film.

The indirect methods of preparations for special studies require preparing special subtle casts (replicas) from slice surface or from fracture of test sample. The replica material has to be able to accurately reproduce the features of micro-relief, to be unstructured, chemically stable, and transparent enough, to subtly scatter electrons and to be separated from the sample surface without being destroyed. The replica method is used:

- To study the relief of massive objects which are opaque to the image-forming electrons;
- For non-conductive samples (the study of such materials in electronic microscopy is complicated because of surface charge accumulation);
- For studying the structure of discharges accumulated on replica.

The replicas are obtained from the sample surface with a good topography featuring the sample microstructure. The most common method of replica preparation is method of thin (mostly, carbon) film deposition.

This year, *Leica Microsystems* has introduced a new generation of coaters for replica preparation: two instruments of *Leica* EM ACE (Advanced Coating Experience for Electron Microscope) series: EM ACE 200 and EM ACE 600.

These instruments are manufactured with taking into account all the requirements for sample preparation, from spray coating to cryofracture. The manufacturer objective is absolutely clear: to make the deposition process simple, fast, and reliable to achieve the optimal sample image in electron microscope.

Application of conductive layer to the sample prevents the charge accumulation, reduces the thermal damages, and improves the secondary electron signal required for topographical studies in SEM. The thin carbon layers which are transparent and conductive with respect to electrons are required for X-ray microanalysis to support the specimens on grids and the backup replicas to be displayed by TEM method. The coating technique depends primarily on resolution and field of application. The *Leica* EM ACE instrument family provides a perfect result in any field.

The *Leica* EM ACE tools are manufactured in two variants: *Leica* EM ACE 200 for spraying in the low vacuum (up to 7×10^{-3} mbar) for conventional SEM and TEM analysis and *Leica* EM ACE 600 for spraying in the high vacuum (up to 2×10^{-7} mbar) for the analysis of high-resolution TEM and FE-SEM. The latter has an optional cryovacuum system for sample transfer. Both devices are ergonomic, compact, and have an automatic control system. The program settings can be given via touch multiplayer menu. The device can be programmed for performing the special laboratory tasks.

The ***Leica* EM ACE 200** (Fig. 1) is a high-quality desktop device producing conductive metal or carbon coating required for electron microscopy. This fully automated model can be configured as a sputter coater or as a carbon thread evaporation coater. If both methods are required this instrument can combine them due to the interchangeable heads. The additional options include:

- Planetary rotation of table: for equidistribution of coating material on fractured samples;
- Glow discharge: for the manufacture of hydrophilic grids for TEM;
- Exchangeable shielding: for easy cleaning of chamber.

The device allows the user to apply the highly reproducible, smooth, and gently deposited carbon

Fig. 1. *Leica* EM ACE 200. General view

Fig. 2. *Leica* EM ACE 600. General view

Fig. 3. *Leica* EM ACE 600 with cryo option

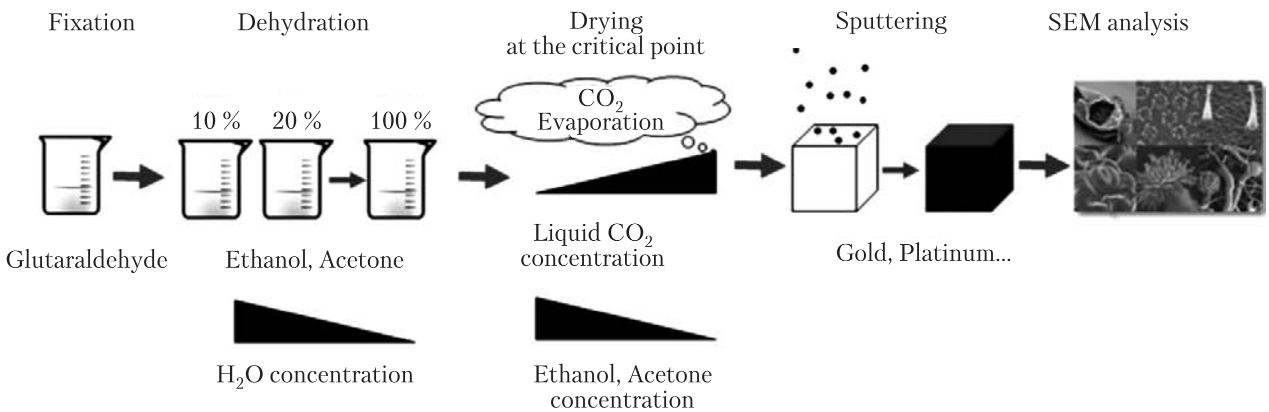

Fig. 4. Stages of sample preparation for SEM analysis

Fig. 5. Automatic line for sample preparation

layers having a thickness of up to 40 nm with fixed borders due to a unique process control system with the use of software. The carbon thread evaporates using the impulse procedure with a continuous monitoring of thickness (quartz measurement).

The ***Leica* EM ACE 600** (Fig. 2) is a perfect versatile high vacuum film deposition system designed to produce very thin, fine-grained and conductive metal and carbon coatings for highest resolution analysis, as required for FE-SEM and TEM applications. This fully automatic tabletop unit includes a built oil-free pumping system, a quartz crystal to measure the film thickness, and a three-axis motorized rotatable table with adjustable tilt and height. This high vacuum coater can be configured for the following methods:

- Sputtering;
- Carbon thread evaporation;
- Carbon rod evaporation (with an option for thermal evaporation);
- E-beam evaporation;
- Glow discharge;
- The *Leica* EM VCT 100 vacuum cryo transfer system is adaptable to the methods of contamination-free cryo SEM sample preparation (cryo coating, cryo shearing, double replica, cryo drying, and transfer) with the help of VCT-shuttle.

The ***Leica* EM ACE 600** equipped with cryo transfer system Leica EM VCT 100 (Fig. 3) is the ideal solution for sample preparation in cryo-SEM conditions with complete control over environmental factors. If the system is configured to use the cryo methods, the tables used at ambient temperature are exchanged for the cryo tables. The LED light source illuminating the chamber allows the user to observe the whole process of sputtering.

The functions of *Leica* EM ACE 600 with *Leica* EM VCT 100 Cryosystem include:

- Achieving a high vacuum up to 2×10^{-7} mbar using a cryopump;
- Temperature control for cryo shearing and cryo drying;

- Adjustable height of motor-driven shearing knife;
- Double replication;
- Setting of auxiliary parameters and automatic run of all sputtering cycles;
- Steady cryo conditions for the sample due to vacuum cryo transfer.

Among the problems related to study of biological sample morphology there is preservation of sample structure for SEM study. The samples must be dry in order to be compatible with each other in the microscope vacuum. The water molecules break the vacuum and, consequently, distort the image. They also cause an extensive deformation or destruction of structures during the study directly under electron microscope. The water has a higher surface tension as compared with the air. When crossing the liquid-vapor interface during evaporation (air drying) the tangential forces caused by surface tension may affect the nano- and microstructures of the sample.

Drying at the critical point is the most advanced method for preserving the sample morphology. At the critical point, the physical properties of the liquid and the gas are indistinguishable. If the subject of research at the critical point can be liquiated or gasified without transition between the liquid and the gaseous phase, any destruction is excluded. The sample dehydration at the critical point is impossible unless temperature is under 374 °C and pressure is under 229 bars. In this case any biological sample is destroyed. To overcome this problem water can be replaced by liquid carbon dioxide (CO_2) whose critical point is 31 °C and 74 bars. This procedure fits all the biological applications and is easy to perform from technical point of view.

However, CO_2 has a serious drawback as a transitional fluid inasmuch as it is insoluble in water and is inmiscible with it. Therefore, water must be replaced by methanol or acetone, i.e. a substance that can be miscible with both water and carbon dioxide CO_2. Both liquids cannot be used for drying at the critical point, because they have high critical temperatures (ethanol: P_c = 60 bar/T_c = 241 °C; acetone: P_c = 46 bar/T_c = 235 °C).

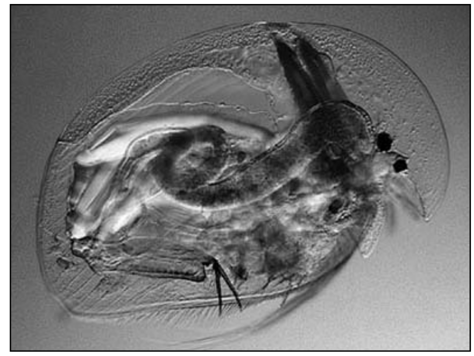

Fig. 6. Daphnia (water flea *Daphnia sp.*). General view

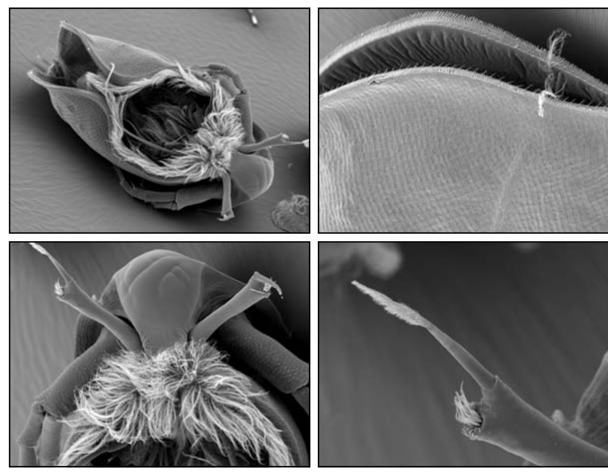

Fig. 7. Daphnia (water flea *Daphnia sp.*). SEM image

After substitution of water with intermediate liquid the latter is replaced by liquid carbon dioxide which is brought to the critical point of transition into gaseous phase under dropping pressure at the constant critical temperature.

Thus, for the preparation of biological sample containing water in order to preserve the sample structure in vacuum conditions under scanning electron microscope the following stages of preparation shall be made (Figs. 4, 5):

1) Fixation: cross-linking of proteins to increase mechanical and thermal stability;

2) Dehydration: an increase in concentration of displacement fluid leads to substitution of water in the sample (the process at the first two stages can

be automated with the help of *Leica* EM TP automatic processor for fixation and dehydration);

3) Substitution of displacement fluid with liquid carbon dioxide CO_2 in the sample with subsequent drying at the critical point (at the third stage of sample preparation, *Leica* EM SPD300 machine for drying at the critical point can be used to automate the process);

4) Sputtering of conductive coatings for SEM analysis.

Leica Microsystems offers a complete range of instruments for EM sample preparation and new coaters. The *Leica* EM ACE appliances fit perfectly the research process. The only way to achieve optimum results in high resolution electron microscopy is to meet strictly and carefully each step of sample preparation procedure, which guarantees the highest quality of samples.

In Figures 6 and 7, there are given examples of *Leica Microsystems* device application: the results are kindly provided by *D. Gruber*, University of Vienna, Austria: a general view of Daphnia (water flea) and images under scanning electron microscope with different magnifications.

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ПІДГОТОВКА ЗРАЗКІВ ДЛЯ ПРОСВІЧУЮЧОЇ ТА СКАНУЮЧОЇ ЕЛЕКТРОННОЇ МІКРОСКОПІЇ: НОВІ УСТАНОВКИ ВІД LEICA MICROSYSTEMS ДЛЯ НАНЕСЕННЯ ПОКРИТТІВ

Надана інформація про нові настільні установки від Leica Microsystems для нанесення струмопровідних покриттів в вакуумі. Представлена інформація про технічні характеристики та область застосування таких установок.

Ключові слова: нанотехнології, електронна мікроскопія, аналіз мікроструктур, репліка, автоматичне управління, рівень вакууму.

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ПОДГОТОВКА ОБРАЗЦОВ ДЛЯ ПРОСВЕЧИВАЮЩЕЙ И СКАНИРУЮЩЕЙ ЭЛЕКТРОННОЙ МИКРОСКОПИИ: НОВЫЕ УСТАНОВКИ ОТ LEICA MICROSYSTEMS ДЛЯ НАНЕСЕНИЯ ПОКРЫТИЙ

Представлена информация о новых настольных установках от Leica Microsystems для нанесения токопроводящих покрытий в вакууме. Представлена информация о технических характеристиках и области использования данных установок.

Ключевые слова: нанотехнологии, электронная микроскопия, анализ микроструктур, реплика, автоматическое управление, уровень вакуума.

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