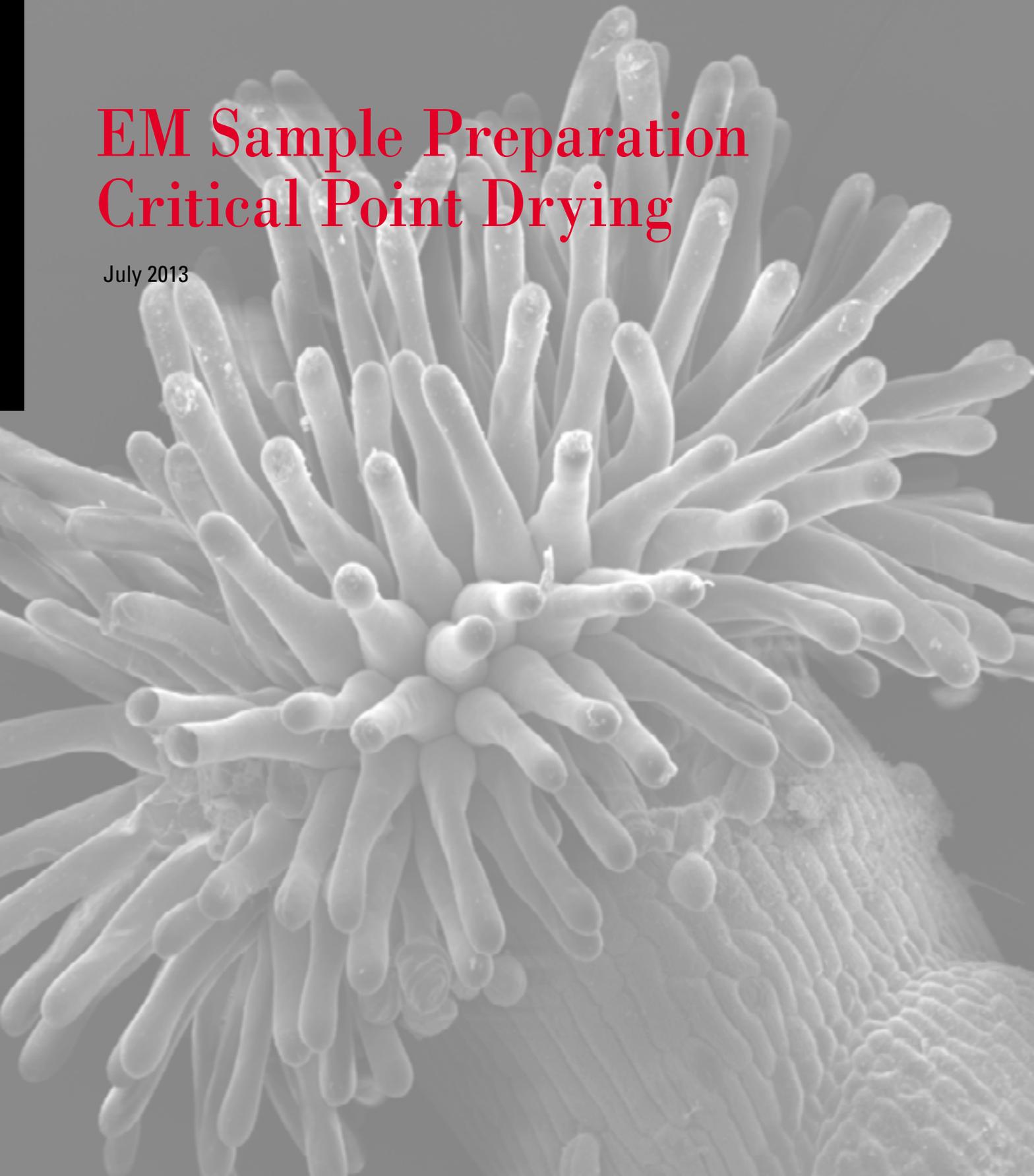


Living up to Life

*Leica*  
MICROSYSTEMS

# EM Sample Preparation Critical Point Drying

July 2013

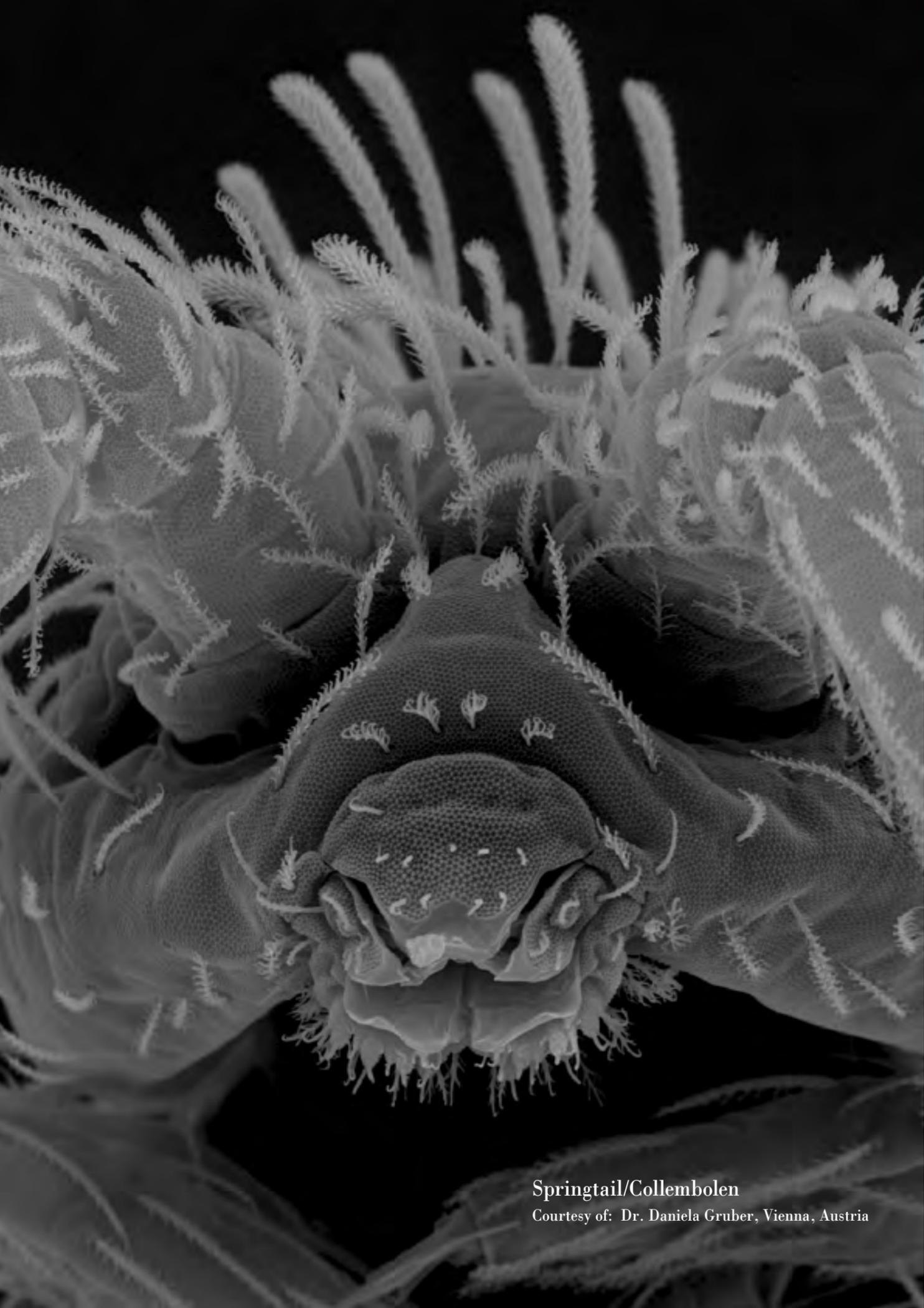


## Critical Point Drying

One of the uses of the Scanning Electron Microscope (SEM) is in the study of surface morphology in biological applications which requires the preservation of the surface details of a specimen. Samples for Electron Microscopy (EM) imaging need to be dried in order to be compatible with the vacuum in the microscope. The presence of water molecules will disturb the vacuum and with it the imaging. It will also cause massive deformation or collapse of the structures under investigation (see “comparison between air and critical point drying”). Water has a high surface tension to air. Crossing the interfaces from liquid to gaseous phase during evaporation (air drying) the tangential forces caused by the surface tension can have an effect on the nano and micro structures of the specimen.

To preserve sample morphology, critical point drying is the state of the art method (see “pressure / temperature phase diagram for CO<sub>2</sub>”). At the critical point, the physical characteristics of liquids and gases are not distinguishable. Compounds which are at the critical point can be converted into the liquid or gaseous phase without crossing the interfaces between liquid and gas, thus avoiding the damaging effects. Dehydration of samples using the critical point of water is not feasible since it lies at 374 °C and 229 bar where any biological sample would be destroyed. To overcome this problem, water can be replaced against liquid carbon dioxide (CO<sub>2</sub>), whose critical point lies at 31°C and 74 bar and is more appropriate for all biological applications and technically relative easy to maintain.

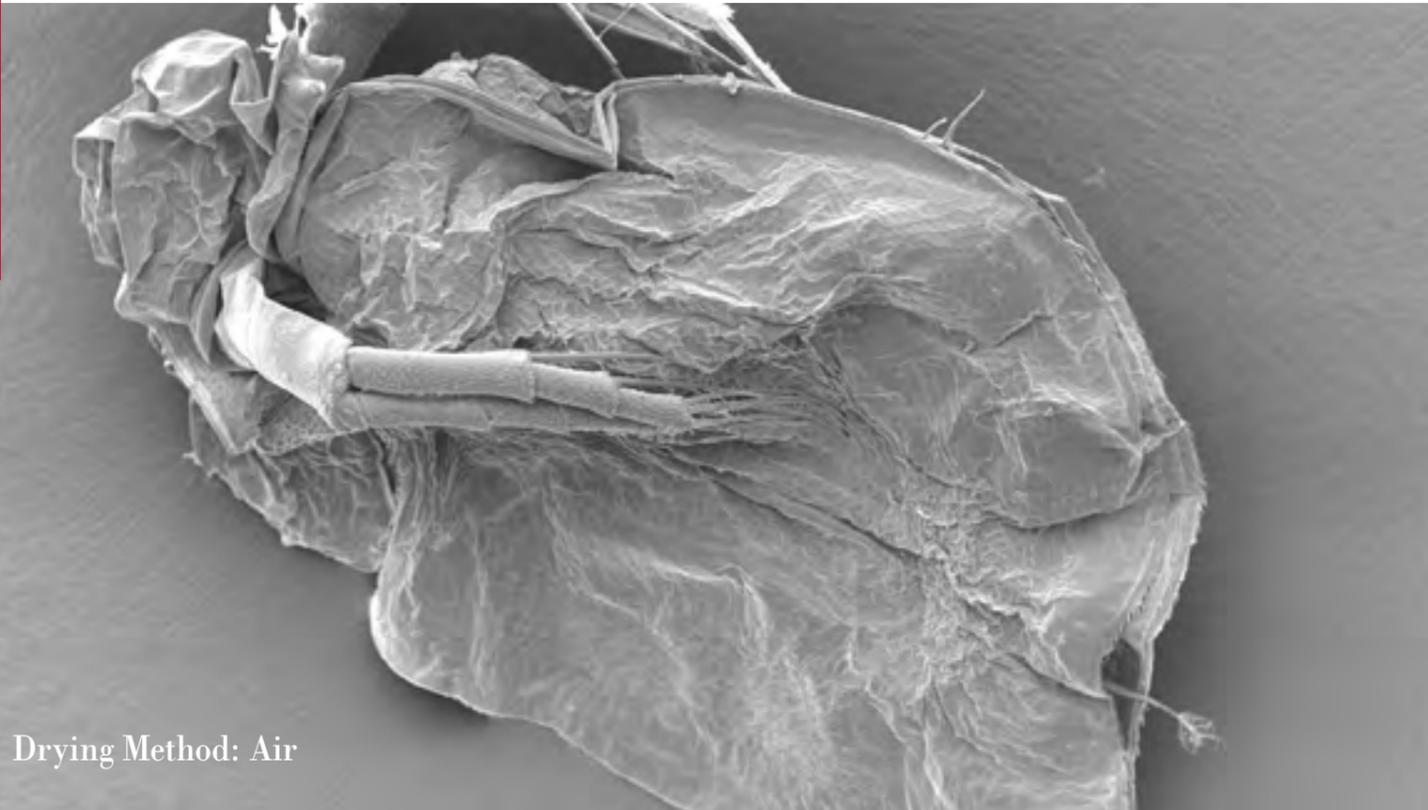
However, CO<sub>2</sub> has one serious disadvantage as a transitional fluid; it is not miscible with water. Therefore, water has to be replaced by exchange fluids like ethanol or acetone which are miscible in both water and liquid CO<sub>2</sub>. Both these exchange fluids cannot be used for critical point drying due to their high critical point temperatures (Ethanol: Pc 60 bar / Tc 241 °C; Acetone: Pc 46 bar / Tc 235 °C). After replacing water with an exchange fluid in a pre-critical point drying step and in turn replacing this exchange fluid with liquid CO<sub>2</sub>, the liquid CO<sub>2</sub> is brought to its critical point and converted to the gaseous phase by decreasing the pressure at constant critical point temperature.



Springtail/Collembolen

Courtesy of: Dr. Daniela Gruber, Vienna, Austria

# Comparison between Air and Critical Point Drying

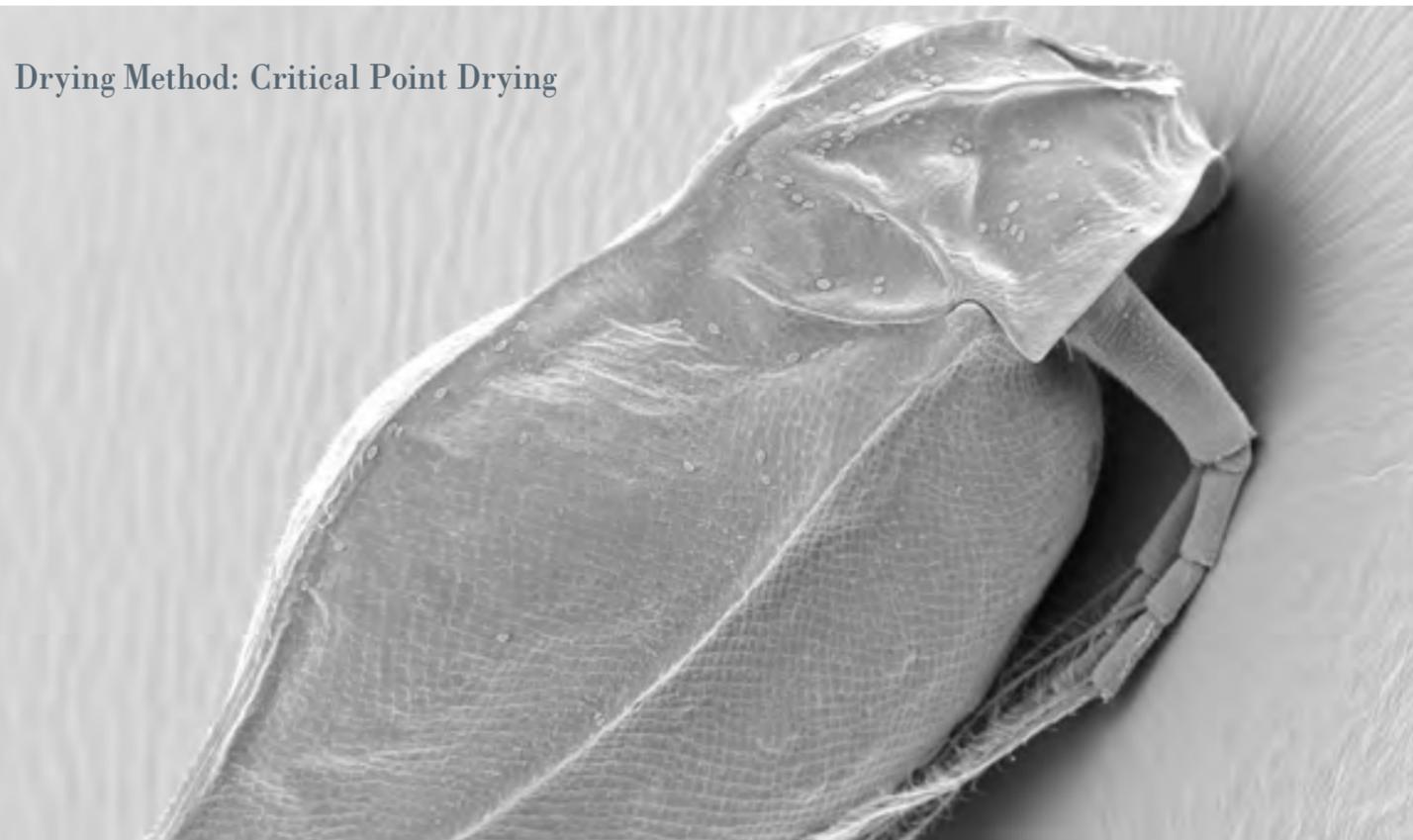


Drying Method: Air

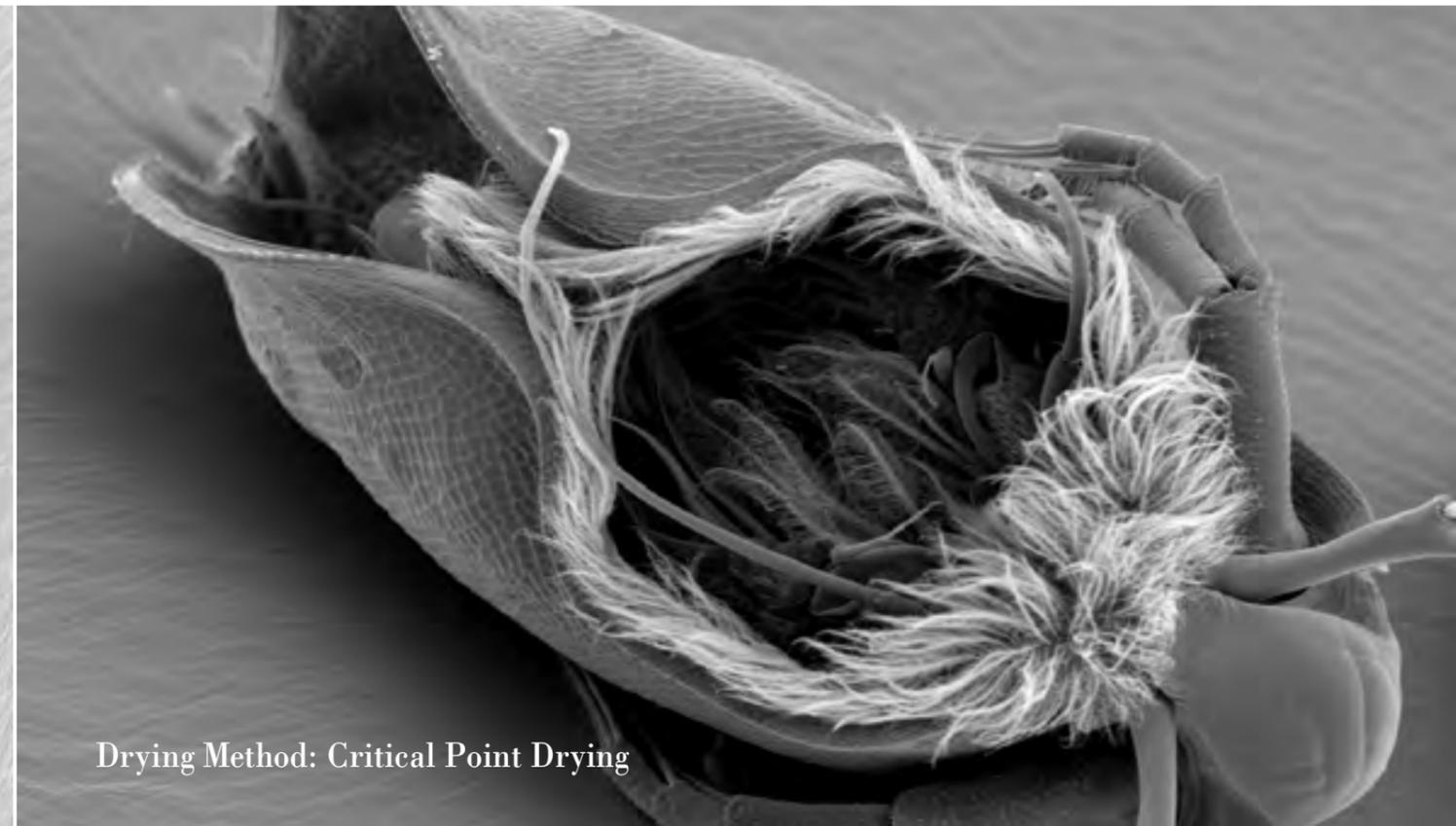


Drying Method: Critical Point Drying

Images Courtesy of: Dr. Daniela Gruber, Core Facility of Cell Imaging and Ultrastructure Research, Vienna Austria



Drying Method: Critical Point Drying

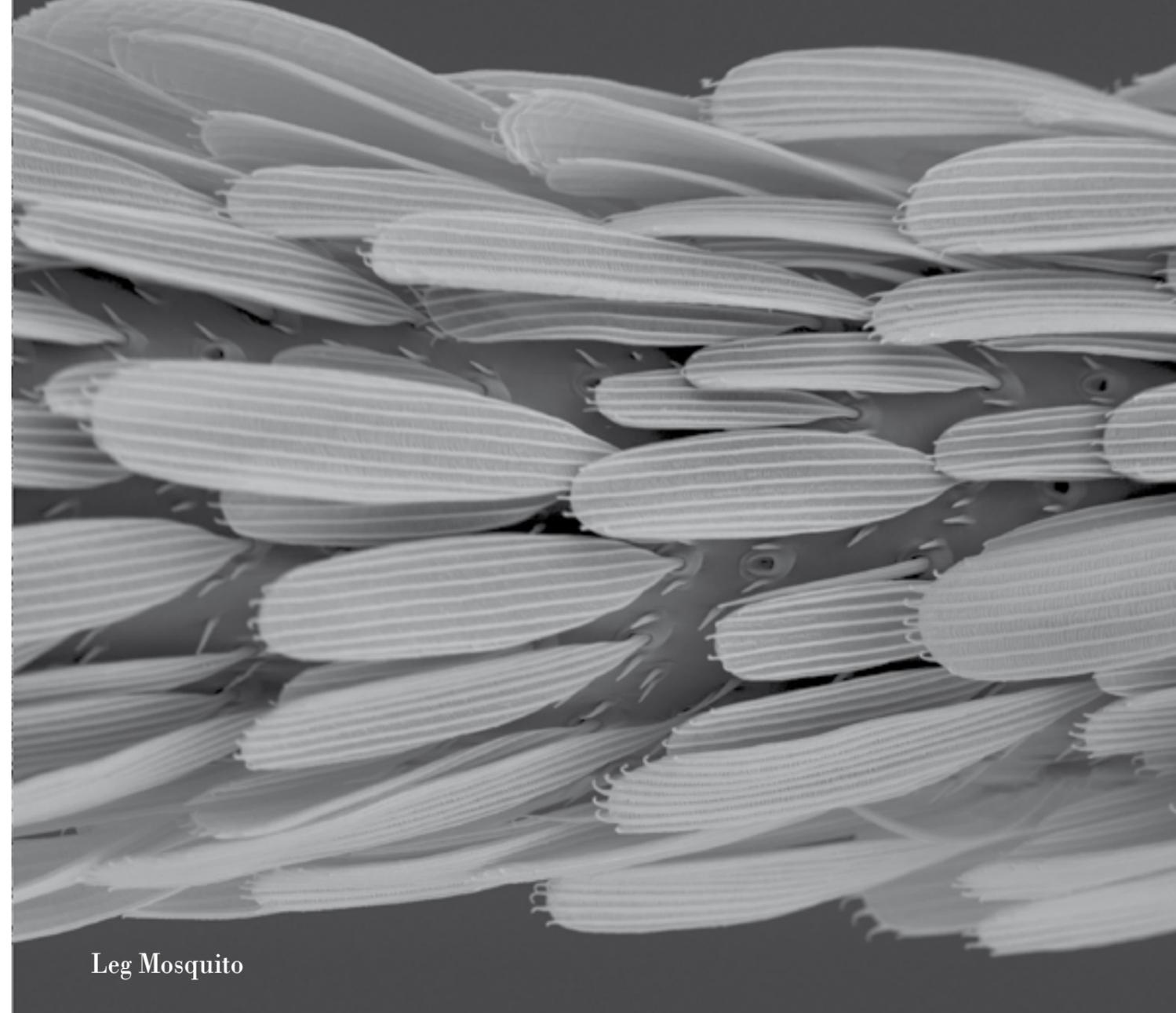


Drying Method: Critical Point Drying

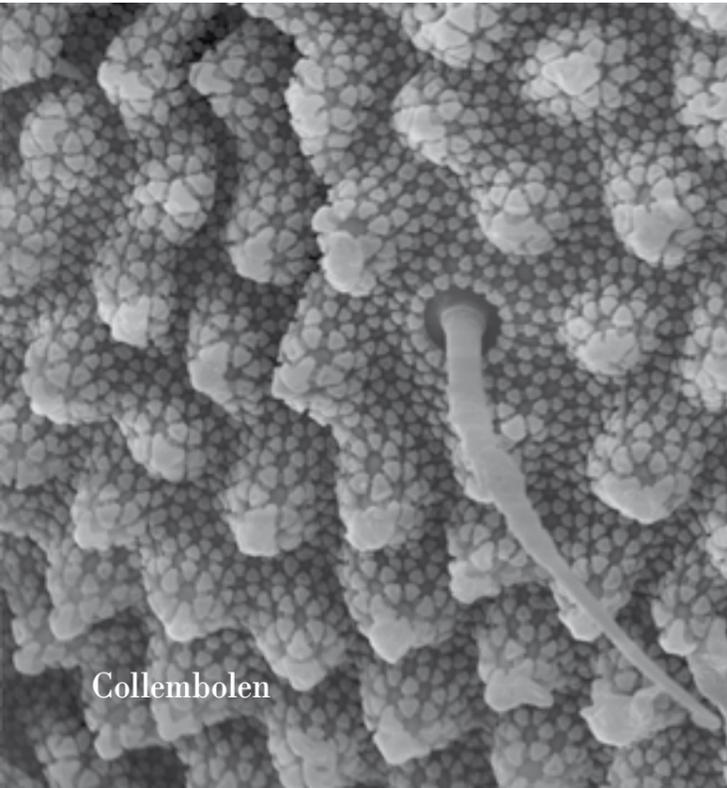
## Applications

For detailed information on applications have a look at the Leica EM CPD300 Application Booklet on our website!

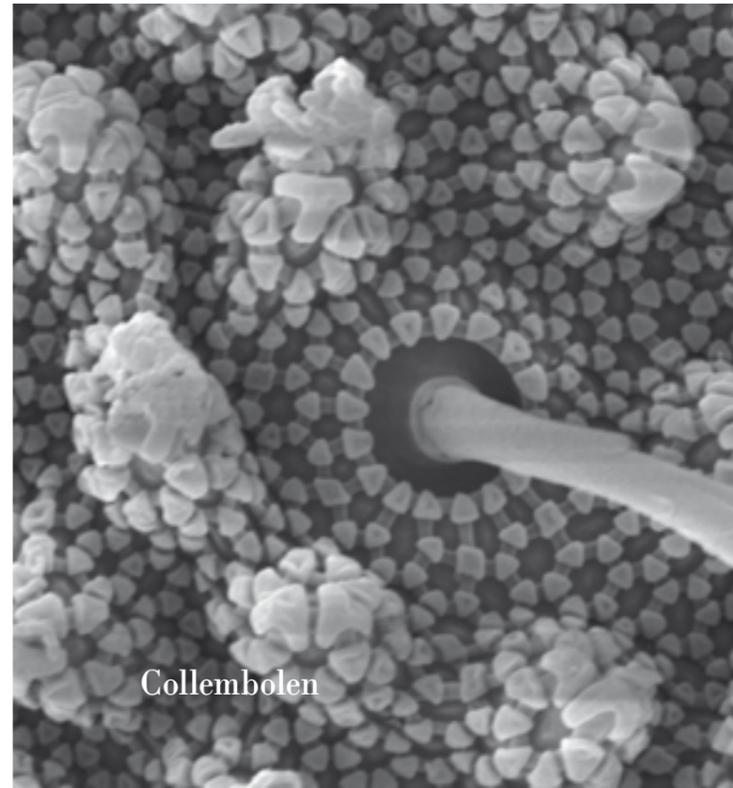
Images Courtesy of: Dr. Daniela Gruber, Core Facility of Cell Imaging and Ultrastructure Research, Vienna Austria



Leg Mosquito



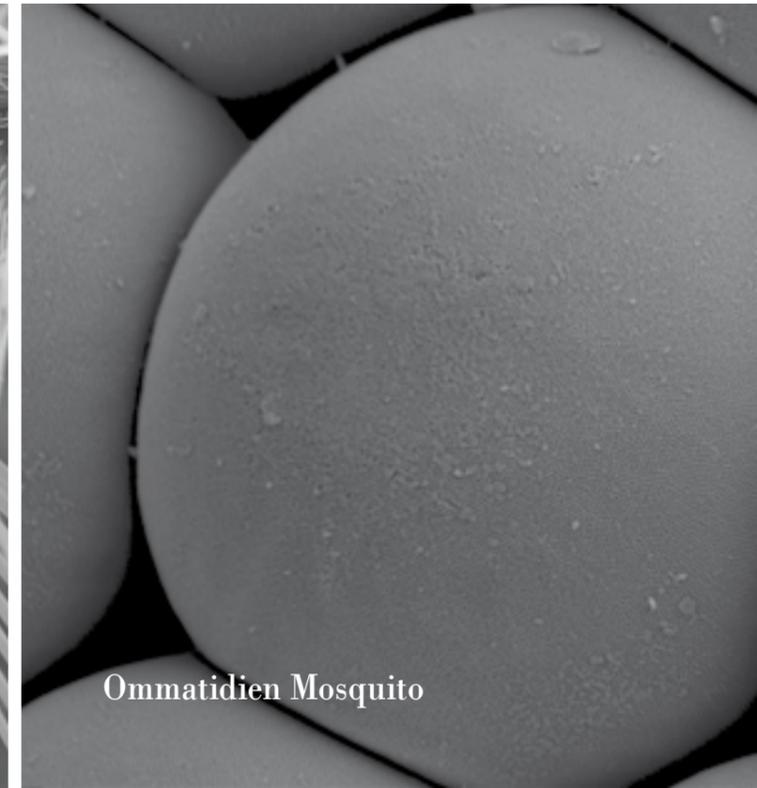
Collembolen



Collembolen



Antenna Mosquito

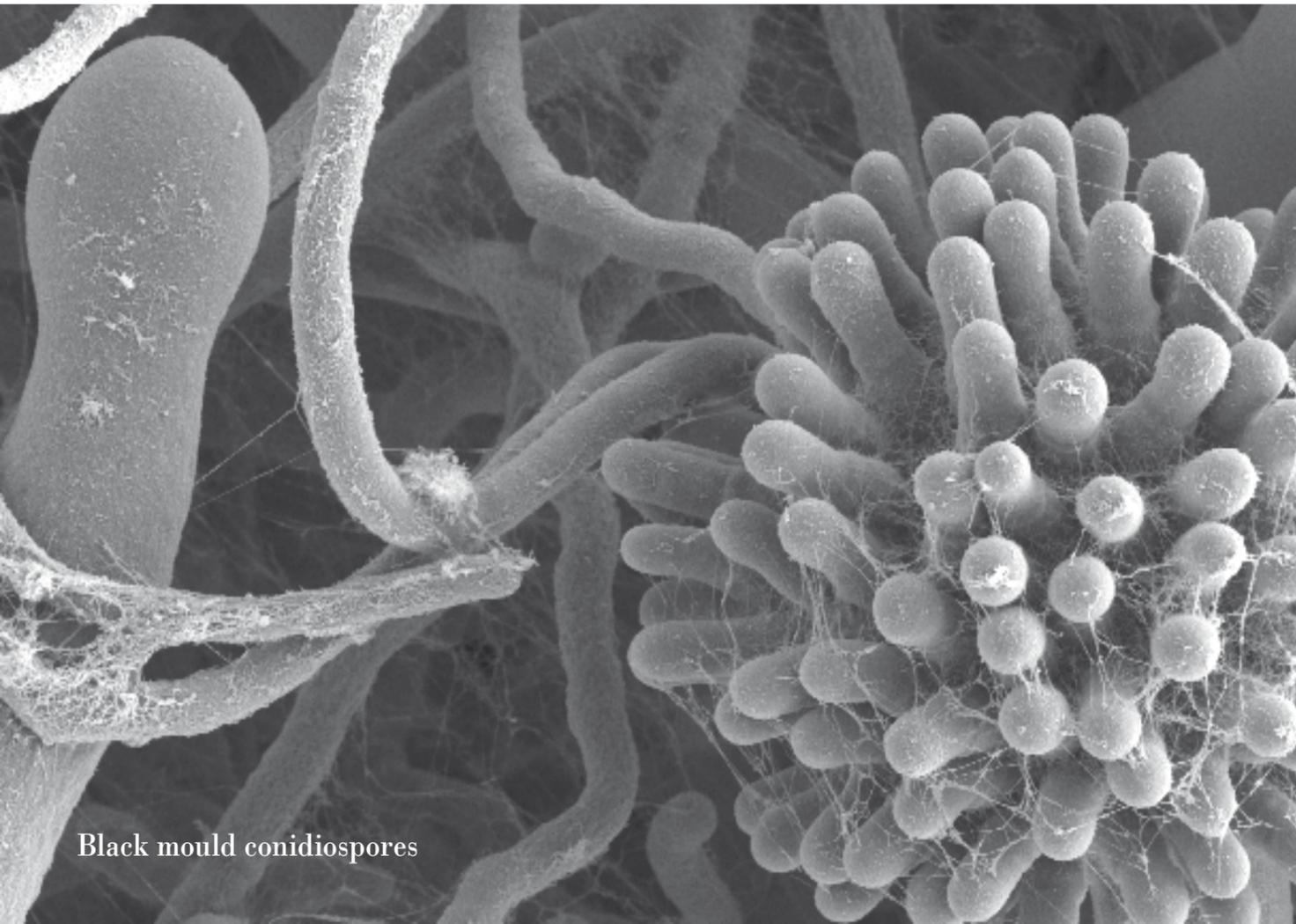


Ommatidien Mosquito



Human Erythrocytes and Lymphocytes

Images Courtesy of: Dr. W. Müller, University of Utrecht, Netherlands



Black mould conidiospores

## Leica EM CPD300

### Automated Critical Point Dryer

The new Leica EM CPD300 dries specimens such as pollen, tissue, plants, insects, etc. as well as industrial samples, for example Micro Electro Mechanical Systems (MEMS), in a fully automated and controlled process. This automated, controlled technique leads to perfect, reproducible results and ensures the same sample quality from every run.



The statement by Ernst Leitz in 1907, “*With the User, For the User,*” describes the fruitful collaboration with end users and driving force of innovation at Leica Microsystems. We have developed five brand values to live up to this tradition: Pioneering, High-end Quality, Team Spirit, Dedication to Science, and Continuous Improvement. For us, living up to these values means: **Living up to Life.**

Leica Microsystems operates globally in four divisions, where we rank with the market leaders.

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#### LIFE SCIENCE DIVISION

The Leica Microsystems Life Science Division supports the imaging needs of the scientific community with advanced innovation and technical expertise for the visualization, measurement, and analysis of microstructures. Our strong focus on understanding scientific applications puts Leica Microsystems' customers at the leading edge of science.

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#### INDUSTRY DIVISION

The Leica Microsystems Industry Division's focus is to support customers' pursuit of the highest quality end result. Leica Microsystems provide the best and most innovative imaging systems to see, measure, and analyze the microstructures in routine and research industrial applications, materials science, quality control, forensic science investigation, and educational applications.

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#### BIOSYSTEMS DIVISION

The Leica Microsystems Biosystems Division brings histopathology labs and researchers the highest-quality, most comprehensive product range. From patient to pathologist, the range includes the ideal product for each histology step and high-productivity workflow solutions for the entire lab. With complete histology systems featuring innovative automation and Novocastra™ reagents, Leica Microsystems creates better patient care through rapid turnaround, diagnostic confidence, and close customer collaboration.

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#### MEDICAL DIVISION

The Leica Microsystems Medical Division's focus is to partner with and support surgeons and their care of patients with the highest-quality, most innovative surgical microscope technology today and into the future.