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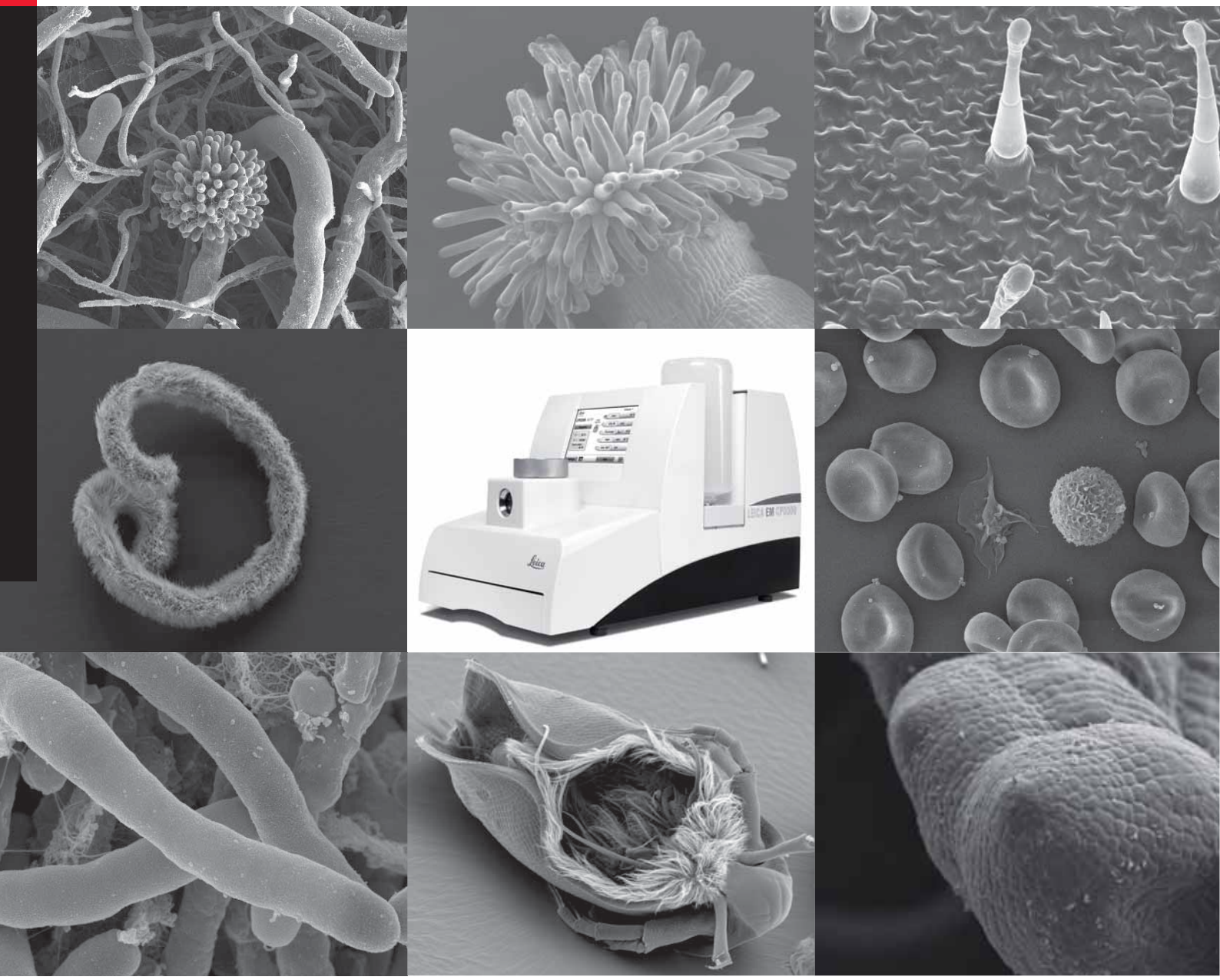

Application Booklet

Leica EM CPD300 Automated Critical Point Dryer

Foreword

This Application Booklet is intended to provide standard protocols to facilitate the optimizing process of critical point drying protocols. The user should always optimize the standard protocol to the sample and experimental conditions.

This Application Booklet includes also information about the principles of critical point drying, a basic description how the Leica EM CPD300 system works as well as hints and tips regarding proper operation.

The Application Booklet is not a user manual replacement. It is essential to read the user manual carefully before beginning any work with the system.

Finally, we would like to thank the following scientists and co-workers for their help to compile this application booklet:

Dr. Chen LiYu, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, China

Dr. Feng Zhenhua, School of Life Sciences and Technology, Tongji University, China

Dr. M. Goldberg and C. Richardson, University of Durham, UK

Mag. D. Gruber, University of Vienna, Austria

Dr. Guo JianSheng, School of Life Sciences and Technology, Tongji University, China

Mag. N. Leisch, University of Vienna, Austria

Dr. W. Müller, University of Utrecht, Netherlands

Dr. K. Rensing, Application Specialist, Leica Microsystems

Dr. Zhang BoTao, Shanghai Jiao Tong University, China

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1. Introduction

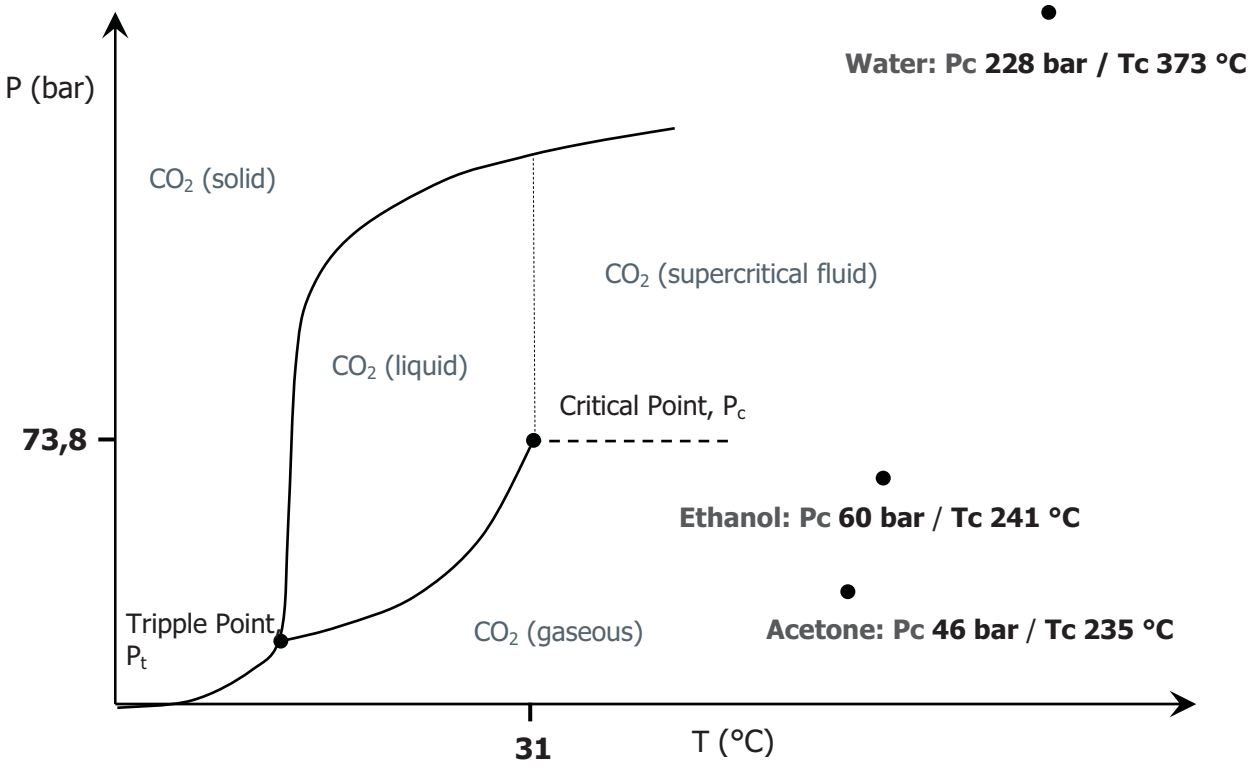
1.1 Critical Point Drying Method

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_2”). At the critical point physical characteristics of liquid and gaseous are not distinguishable. Compounds which are in the critical point can be converted into the liquid or gaseous phase without crossing the interfaces between liquid and gaseous avoiding the damaging effects. The dehydration of the 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_2), 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_2 has one serious disadvantage as 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_2. Both exchange fluids can not 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_2, the liquid CO_2 is brought to its critical point and converted to the gaseous phase by decreasing the pressure at constant critical point temperature.

Pressure Temperature Phase Diagram for CO_2

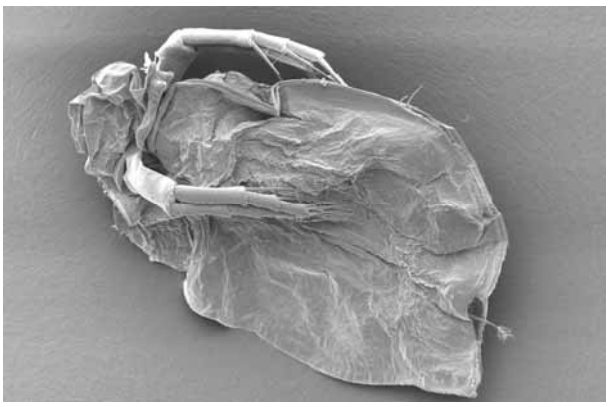


Triple point:
Same physical characteristics of solid, liquid and gaseous.

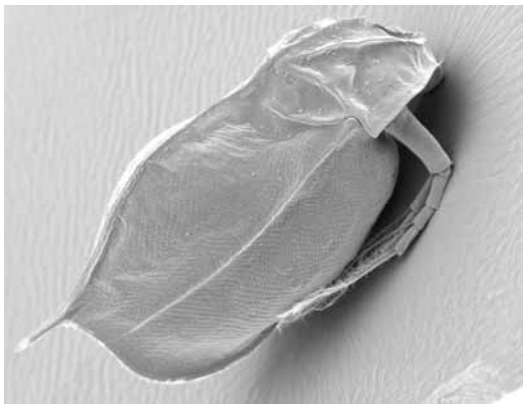
Critical point / Supercritical fluid:
Same physical characteristics of liquid and gaseous.

Comparison between Air and Critical Point Drying

Air dried sample (Water flea)

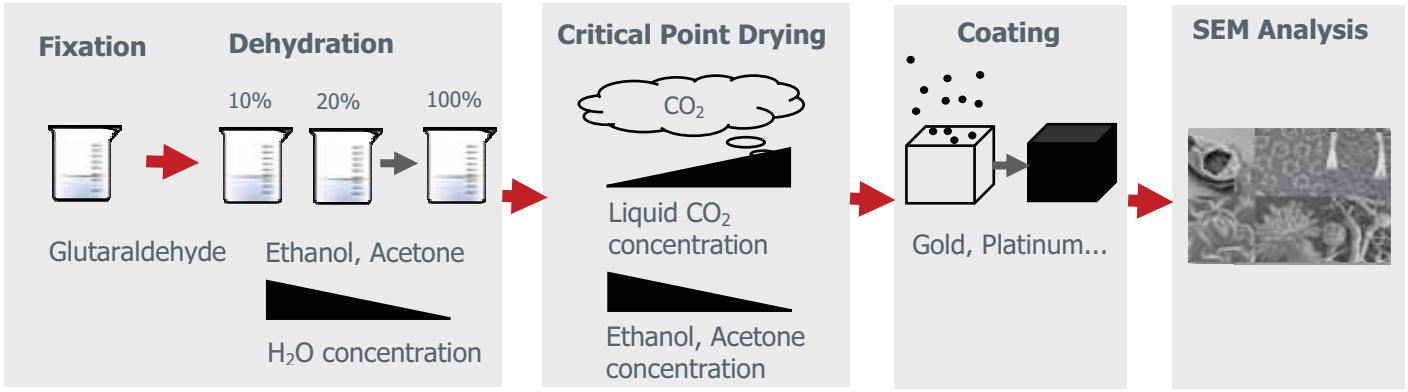


Critical point dried sample (Water flea)



1.2 Workflow for SEM Analysis

Manual Processing:



Automated Processing:

Fixation: Cross links proteins to increase mechanical and thermal stability.

Dehydration: Ascending concentration of exchange fluid replaces water in the sample.

CPD: Replacement of exchange fluid by liquid CO_2 (purging) in the sample, and then critical point drying.

Coating: Makes the sample conductive for SEM Analysis.

1.3 Critical Point Dryer Leica EM CPD300

State of the art Critical Point Drying

- Fully reproducible processes
- Highly reproducible sample preparation
- Possibility to store and retrieve recipes and programs
- Minimized time the user has to interfere with the instrument
- Ease of use by intuitive software and integrated touch screen user interface
- Expected process time calculated and displayed according to selected process parameters
- Increased safety by software controlled cut-off function
- Flexibility in sample size (large variety of sample holders)
- Minimized CO_2-Consumption
- Minimized process time
- Immediate calculation and display of complete process time
- Timer function

1.4 Process Steps during Critical Point Drying with Leica EM CPD300

1. First the samples have to be applied into the pressure chamber of the CPD instrument and the sample must be covered with the exchange fluid to prevent air drying.

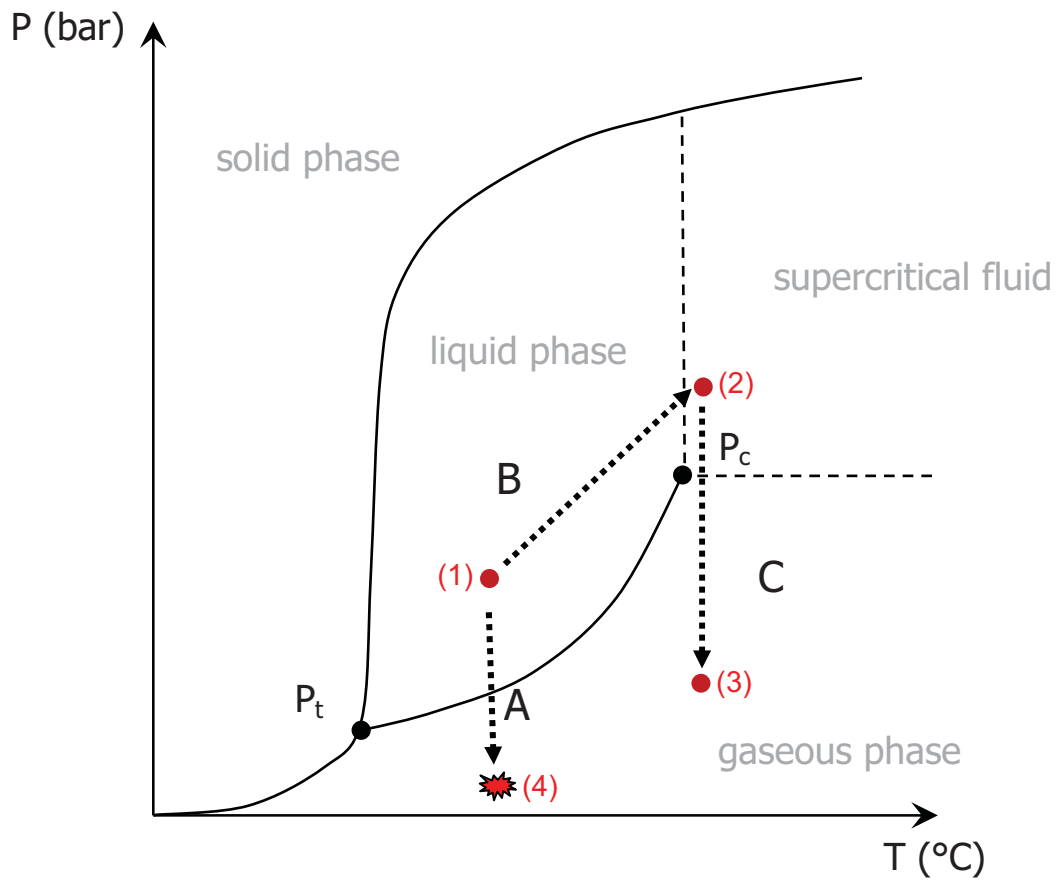
2. Then liquid CO_2 is filled into the pre-cooled pressure chamber. Pre cooling is important to be sure that the CO_2 is liquid during the purging process (1).

3. After CO_2 influx and a certain delay time for mixing, the CO_2-exchange fluid mix is released out of the pressure chamber and new CO_2 is filled. It is important to note that the samples are always covered with liquid to prevent air drying. This is called the purging cycle and has to be done several times depending on the application.

4. After the appropriate number of purging cycles, all the exchange fluids should be replaced by liquid CO_2 and the heating process can be started (2). The Heating process generates supercritical CO_2. The speed of heating can be regulated due to the sample sensitivity.

5. The supercritical CO_2 then forms to gaseous CO_2 by maintaining the temperature constant at 31°C (critical temperature of CO_2) and opening the gas out valve which reduces the pressure in the chamber (3). In this Gas-out step, which is the most crucial step during CPD, the supercritical CO_2 becomes gaseous without crossing the boundary between liquid and gas (4).

Process Diagram Critical Point Drying with CO_2



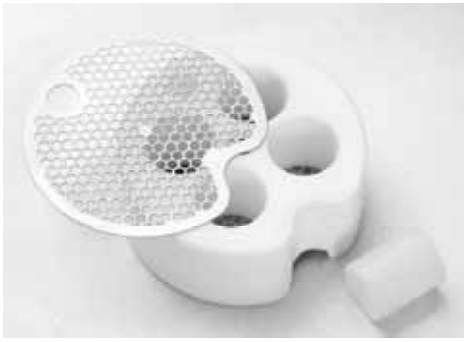
P_c = Critical Point CO_2

P_t = Triple Point CO_2

(4) = Air drying (phase boundary crossing)

(1), (2), (3) = Critical point drying (no phase boundary crossing)

1.5 Sample Holders



Filter Disc and Porous Pot Holder:
4 numbered wells; slot dimension 15 x 21 mm; mesh size 0.5 mm; replaces 50% of chamber volume (1/2 holder).

Recommended use with Filter Discs and Porous Pots. Customized solutions possible, solutions have to fit the slot dimensions.

Fine Mesh Specimen Holder with for 4 fine Mesh Specimen Baskets:
4 numbered wells for fine mesh specimen baskets; mesh size 0.5 mm; replaces 50% of the chamber volume (1/2 holder).

Recommended use with Fine Mesh Specimen baskets. Customized solutions possible, solutions have to fit the slot dimensions.

Cover Slip Holder:
The12 mm dia holder replaces 33% of the chamber volume (1/3 holder).

The 18 mm dia and 22 x 22 mm holders replaces each 50% of the chamber volume (1/2 holders).

Recommended use with cover slips. Customized solutions possible. Solutions have to fit the slot dimensions.

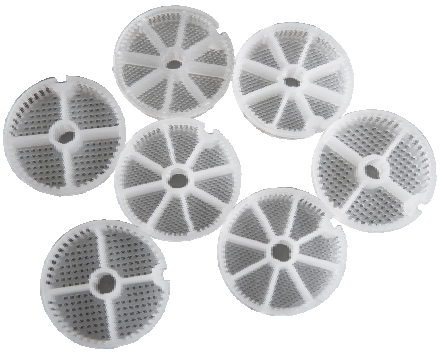
Grid Holder:
32 numbered slots; replaces 16% of chamber volume (1/6 holder).

Recommended use with grids. Customized solutions possible, solutions have to fit the slot dimensions.

TP-Stem Holder of Leica EM CPD300:
Replaces 100% of chamber volume (1/1 holder).
Can not be used with sample transfer basket.

Recommended use with assembled TP-Baskets stem in synergy with Leica EM TP. Customized solutions possible, solutions have to fit the slot dimensions.

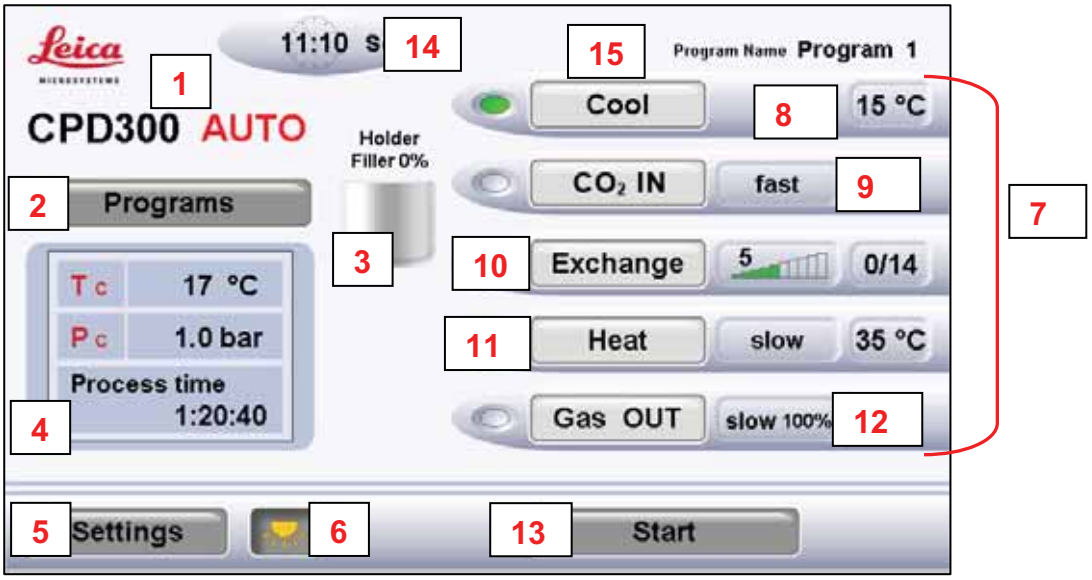
TP Stem with Baskets



TP Baskets

1.6 Short Software Description Leica EM CPD300 auto

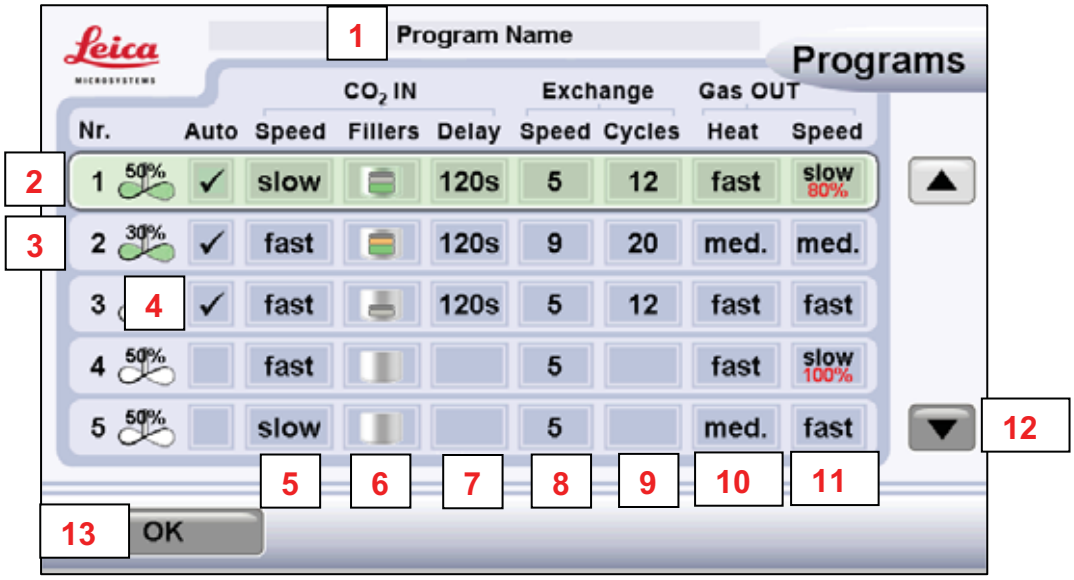
1.6.1 Main Screen Description



Dark grey buttons can be activated, light grey buttons are inactive!

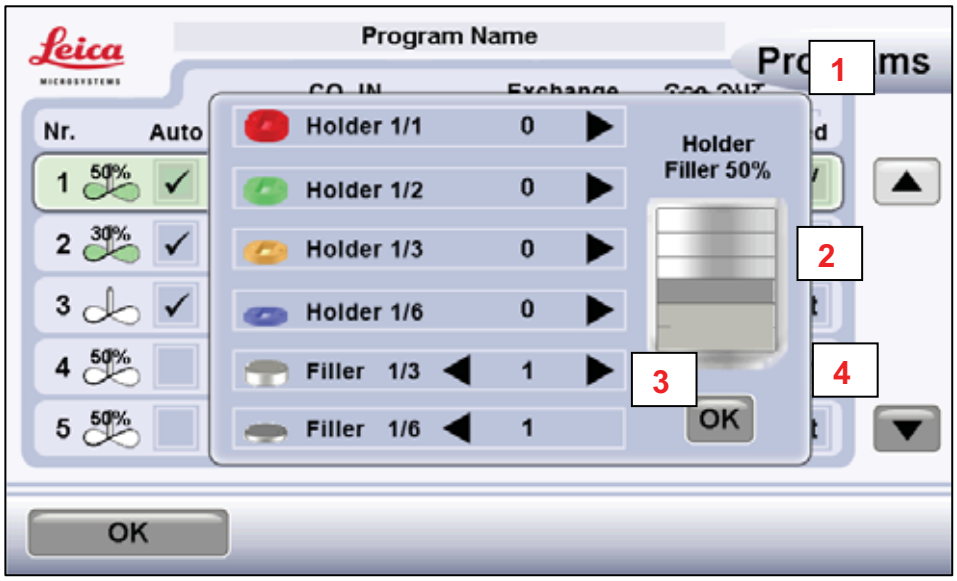
1 Version of the CPD.
2 Switch to program panel (see page 13).
3 Status display of fillers and holder in the sample chamber.
Programmable under programs.
4 Status display temperature, pressure and time to finish the process.
5 Switch to settings.
6 Light on/off
7 Status display of programmed process.
In auto version buttons have no function.
8 Cooling temperature to keep CO_2 fluid (can be changed under settings).
9 CO_2 influx speed in pressure chamber.
Programmable under programs.
10 Exchange speed (1-10) and status of finished exchange cycles.
Programmable under programs.
11 Heating speed and heating temperature for critical point.
Programmable under programs.
12 Status display gas out speed.
Programmable under programs.
13 Process start (after defining program).
14 Timer function.
15 Program name of activated program.

1.6.2 Program Screen



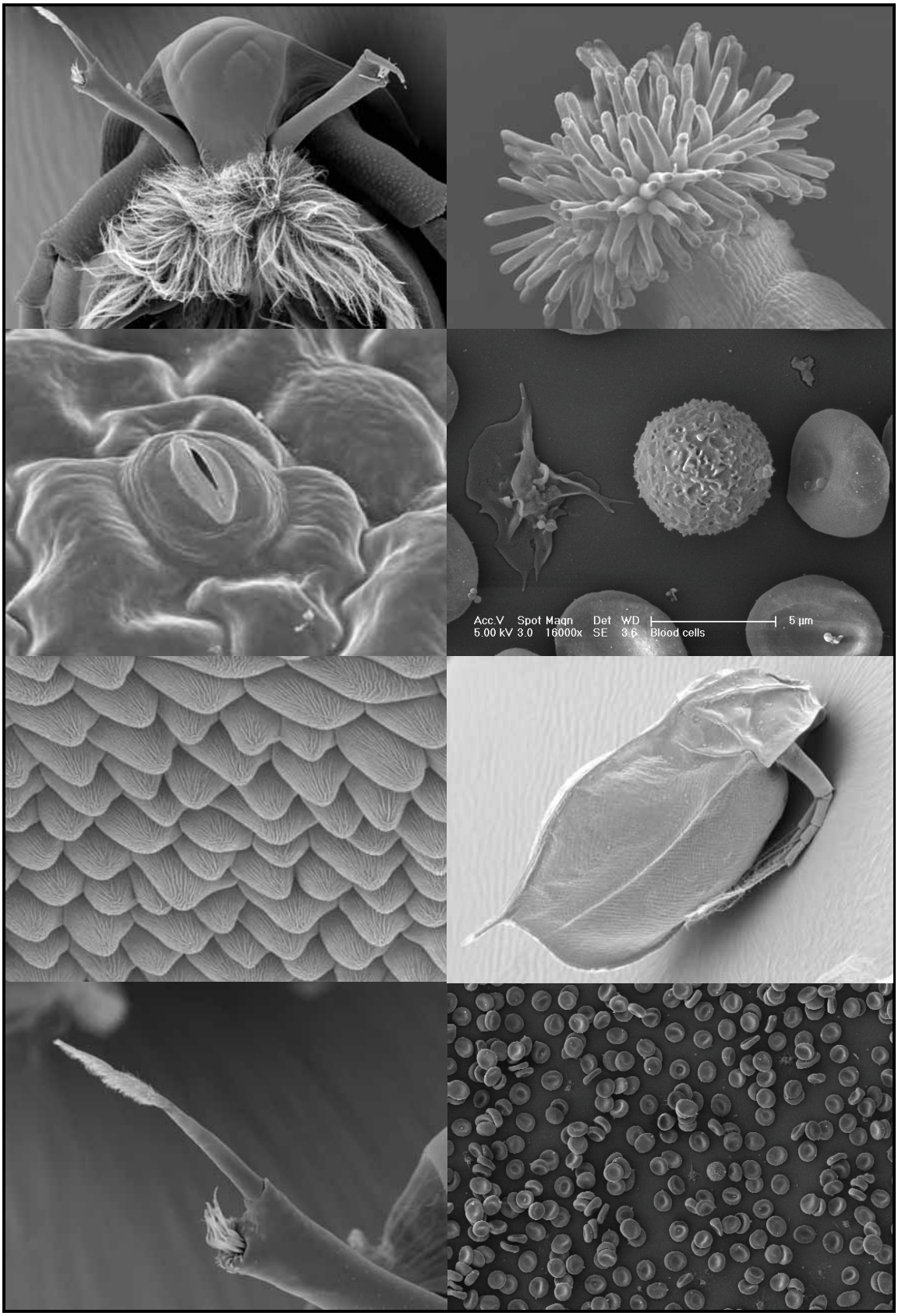
1 Activates key pad to enter program name.
2 Activated program is green marked.
3 Stirrer on / off with speed control.
4 Activation of auto version. If not highlighted manual version is active.
Only selectable in automated version.
5 Sets speed of CO_2 influx in pressure chamber.
Three possibilities: slow, medium, fast.
6 Switch to filler and holder panel. Display of filler and holder status (see page 14).
7 Sets delay time after influx of CO_2 and before starting exchange process.
8 Sets exchange speed from 1-10.
9 Sets exchange cycles. 12 cycles means one chamber volume is completely exchanged. Minimum are 12 cycles.
10 Sets heating speed for critical point. Three possibilities: slow, medium, fast.
11 Sets gas out speed. Possibilities: slow, medium, fast.
Slow speed can be decreased up to 20% of its normal speed.
12 Scrolls programs from 1-10.
13 Confirms activated program. Switch to main screen.

1.6.3 Filler / Holder Panel



1 Filler and holder panel.
2 Status display of fillers and holders.
3 Sets specific holder and fillers. Combination of holders and fillers depends on their volume.
4 Confirms filler and holder setting.

2. Application Protocols



2.1 Plant Protocols

2.1.1 Rice Anther Protocol

Introduction:

Species: Asian Rice *(Oryza sativa)*

Critical point drying of rice anther with subsequent gold coating and SEM analysis.

Procedure:

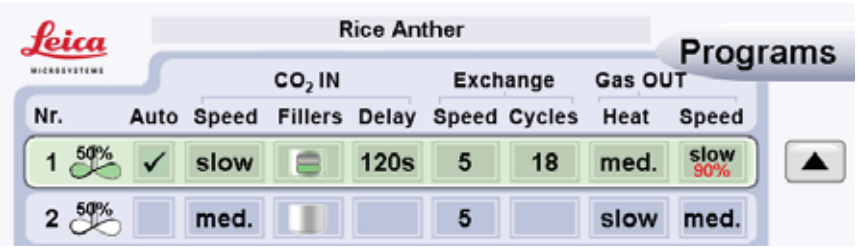
Sample Holder:

Samples were inserted into the 22 mm cover slip holder.

Fixation and Dehydration:

2,5% Glutaraldehyde in 0.1M Sodium Phosphate Buffer, pH 7.2	overnight
0.1M Sodium Phosphate Buffer, pH 7.2	3x 10 min.
Ethanol series: 30%, 50%, 70%, 80%, 90%, 95%, 100%	2x 10 min.

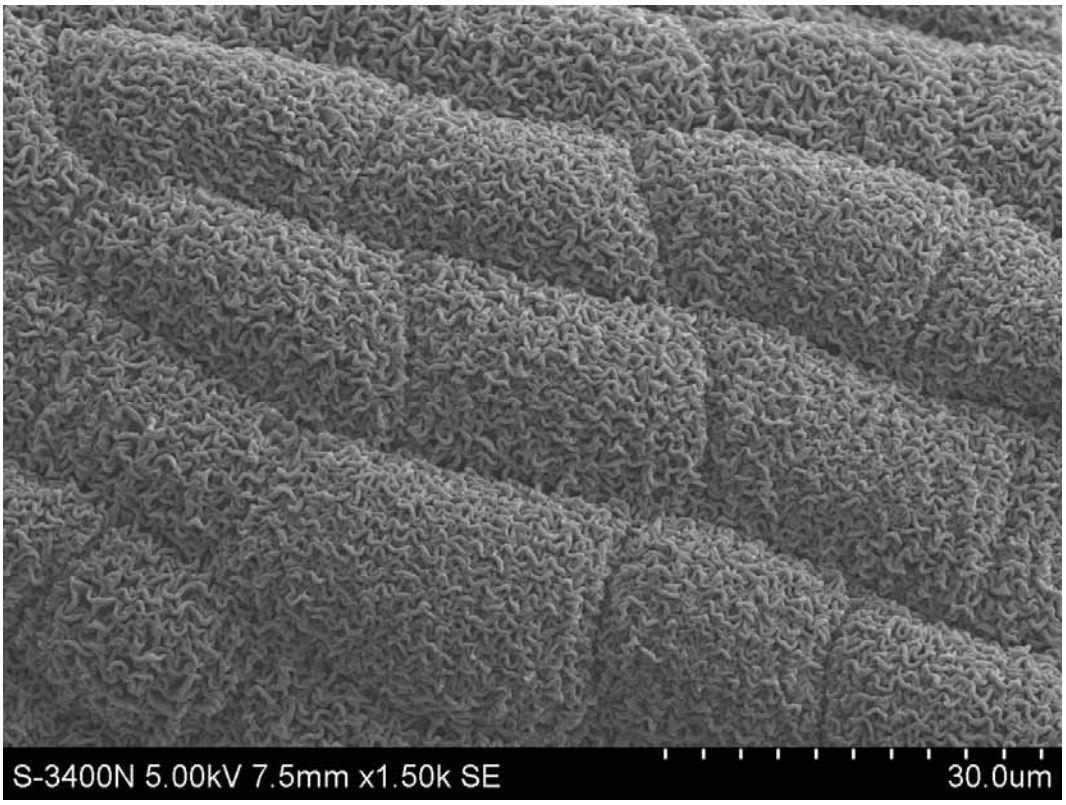
CPD300 auto Program:



Coating:

Gold: 15-20 nm

Results:



Rice anther

Courtesy of Dr. Zhang BoTao, Shanghai Jiao Tong University, China.

2.1.2 Rice Hull Protocol

Introduction:

Species: Asian Rice *(Oryza sativa)*

Critical point drying of rice hull with subsequent gold coating and SEM analysis.

Procedure:

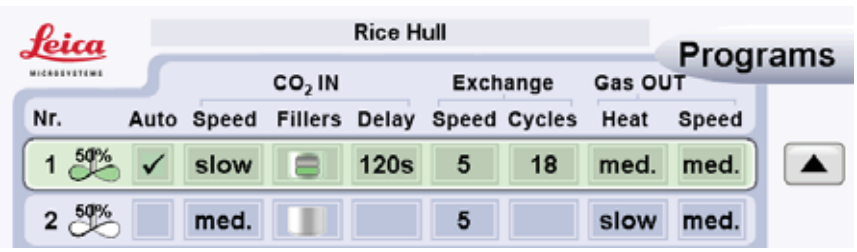
Sample Holder:

Samples were inserted into the 22 mm cover slip holder.

Fixation and Dehydration:

2.5% Glutaraldehyde in 0.1M Sodium Phosphate Buffer, pH 7.2	14 h
0.1M Sodium Phosphate Buffer, pH 7.2	3x 10 min.
Ethanol series: 30%, 50%, 70%, 80%, 90%, 95%, 100%	2x 10 min.

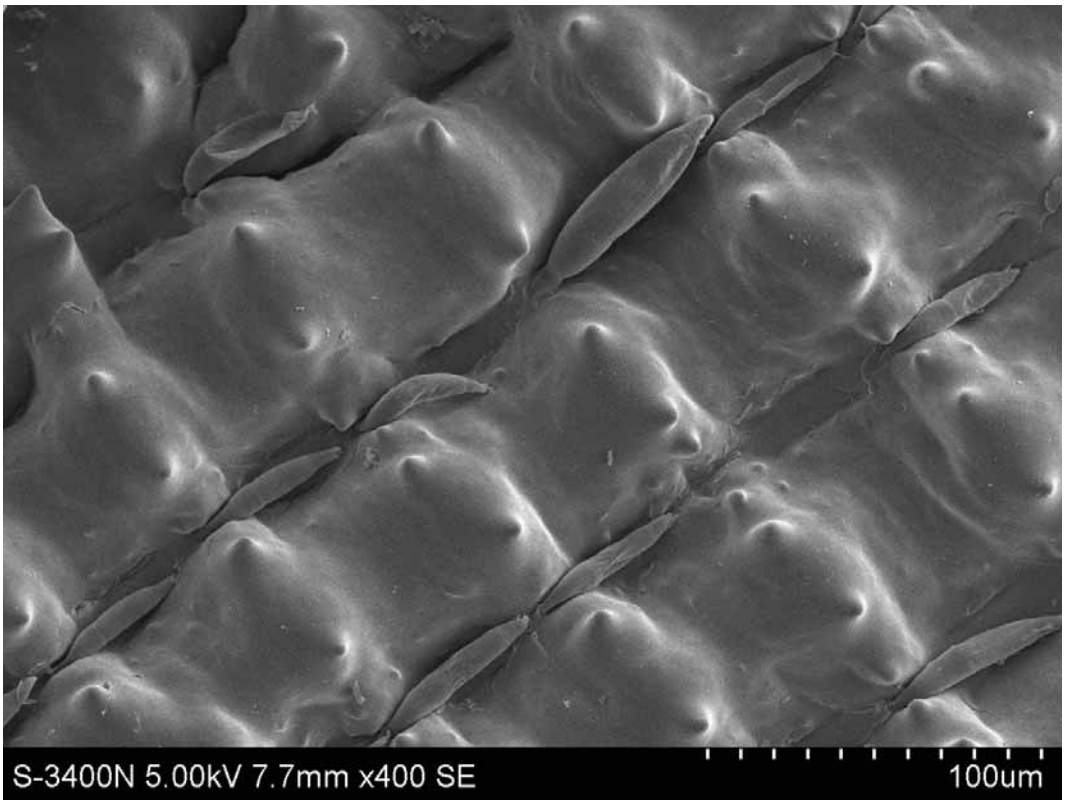
CPD300 auto Program:



Coating:

Gold: 15-20 nm

Results:



Rice Hull

Courtesy of Dr. Zhang BoTao, Shanghai Jiao Tong University, China.

2.1.3 Rice Root Protocol

Introduction:

Species: Asian Rice *(Oryza sativa)*

Critical point drying of rice root with subsequent gold coating and SEM analysis to detect root development stages.

Procedure:

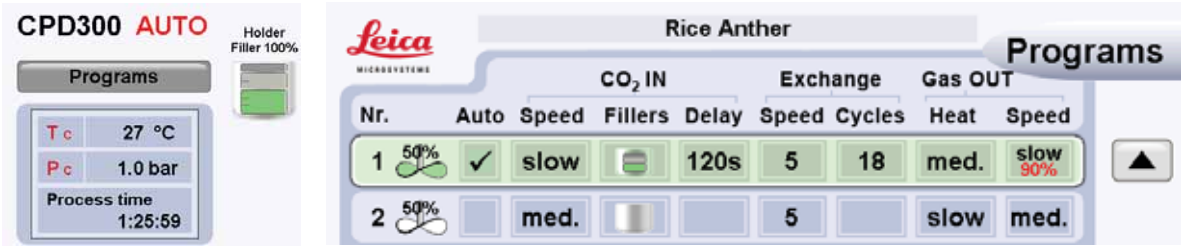
Sample Holder:

Samples were inserted into the 22 mm cover slip holder.

Fixation and Dehydration:

2.5% Glutaraldehyde in 0.1M Sodium Phosphate Buffer, pH 7.2	overnight
0.1M Sodium Phosphate Buffer, pH 7.2	3x 10 min.
Acetone series: 30%, 50%, 70%, 80%, 90%, 95%, 100%	2x 10 min.

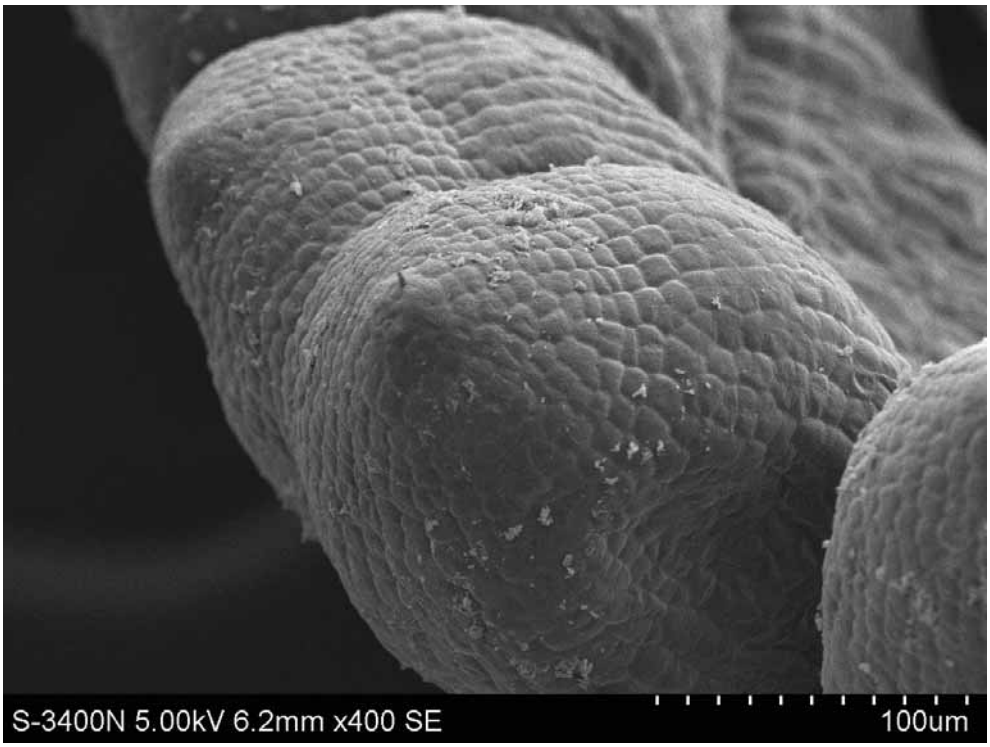
CPD300 auto Program:



Coating:

Gold: 15-20 nm

Results:



The protuberance from rice root explants

Courtesy of Dr. Feng Zhenhua, School of Life Sciences and Technology, Tongji University, China.

2.1.4 Tobacco Leaf Protocol

Introduction:

Species: Tobacco (*Nicotiana tabacum*)

Critical point drying of tobacco leafs with subsequent platinum coating and SEM analysis.

Procedure:

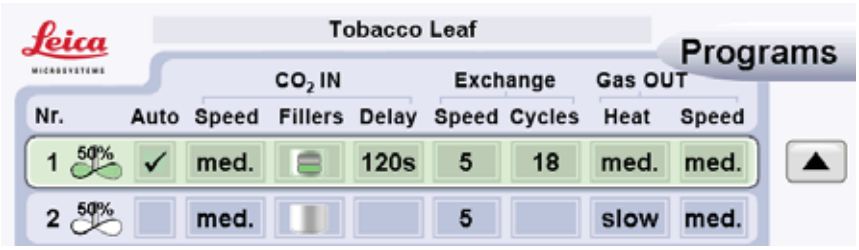
Sample Holder:

Silicon chips containing the samples were placed into the filter discs and porous pots holder.

Fixation and Dehydration:

2% Paraformaldehyde, 2.5% Glutaraldehyde, 0.1M Cacodylate Buffer, pH 7.3	2 h
0.1M Sodium Cacodylate Buffer, pH 7.3	2x 10 min.
1% aqueous OsO_4	1-2 h
Distilled water	3x 10 min.
Ethanol series: 50%, 70%, 95%, 100%	3x 10 min.

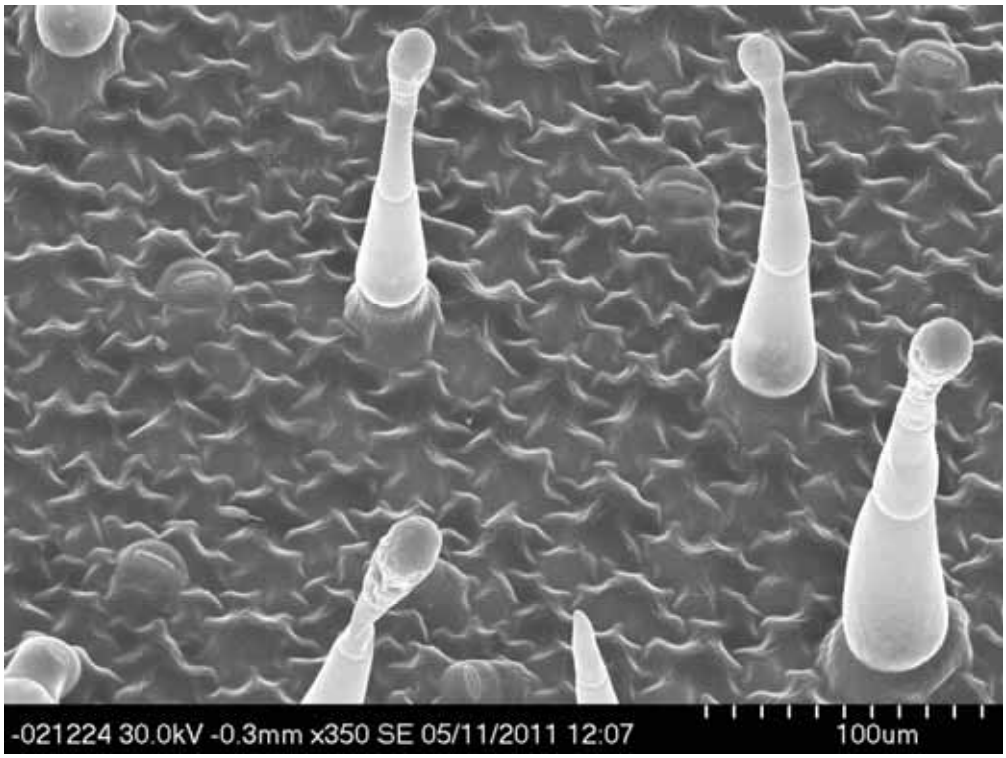
CPD300 auto Program:



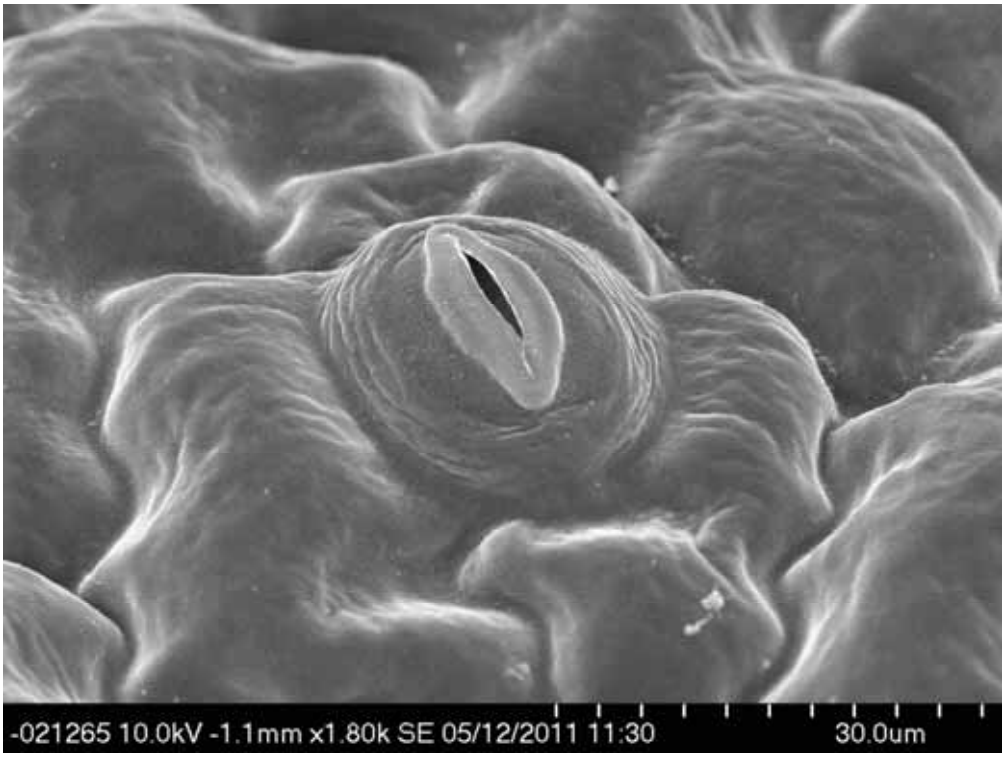
Coating:

Platinum: 3 nm

Results:



Trichomes with Stomata from tobacco leaf



Stomata from tobacco leaf

Courtesy of Dr. M. Goldberg and C. Richardson, University of Durham, UK.

2.1.5 Wall Cress Pod Protocol

Introduction:

Species: Wall Cress (*Arabidopsis thaliana*)

Critical point drying of wall cress pod with subsequent gold coating and SEM analysis.

Procedure:

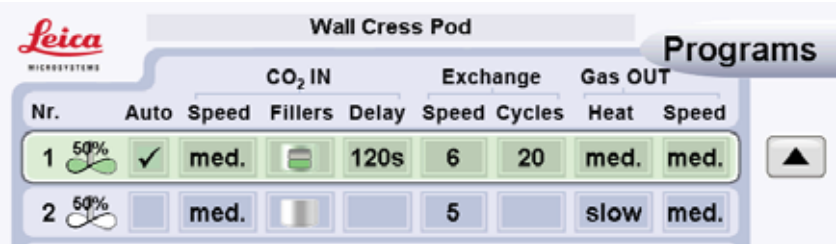
Sample Holder:

Samples were inserted into the 22 mm cover slip holder.

Fixation and Dehydration:

3% Glutaraldehyde in 0.1M Sodium Phosphate Buffer, pH 7.0	overnight
0.1M Sodium Phosphate Buffer, pH 7.0	3x 10 min.
Ethanol series: 30%, 50%, 70%, 80%, 90%, 95%, 100%	2x 10 min.

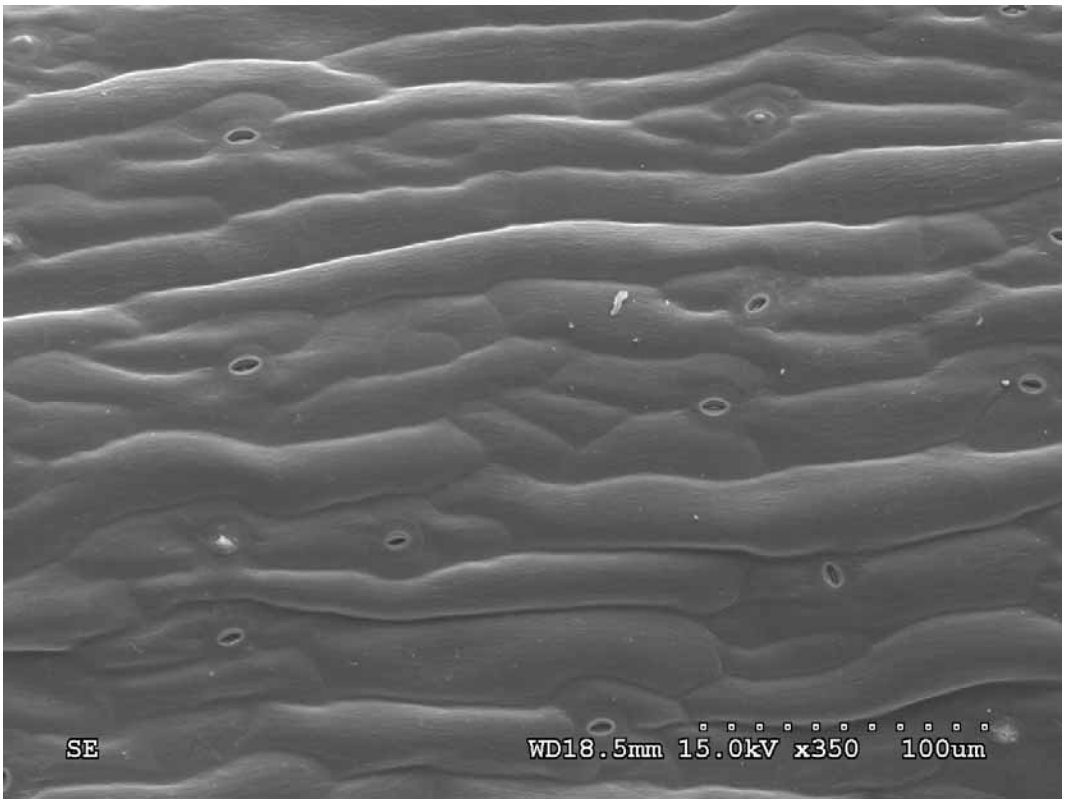
CPD300 auto Program:



Coating:

Gold: 15-20 nm

Results:



Arabidopsis pod

Courtesy of Dr. Chen LiYu, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, China.

2.1.6 Wall Cress Stigma Protocol

Introduction:

Species: Wall Cress (*Arabidopsis thaliana*)

Critical point drying of wall cress stigma with subsequent gold coating and SEM analysis.

Procedure:

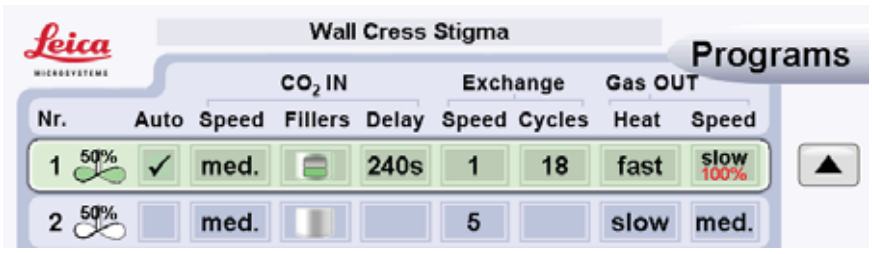
Sample Holder:

Samples were inserted into filter discs and porous pots holder.

Fixation and Dehydration:

2.5% Glutaraldehyde in 0.1M Sodium Cacodylate Buffer, pH 7.3	1x 2 h
0.1 M Sodium Cacodylate Buffer, pH 7.3	3x 10 min.
1% OsO4, in 0.1M Sodium Cacodylate Buffer, pH 7.3	1x 1 h
0.1 M Sodium Cacodylate Buffer, pH 7.3	3x 10 min.
Ethanol series: 30%, 60%, 95%, 100%	3x 10 min.

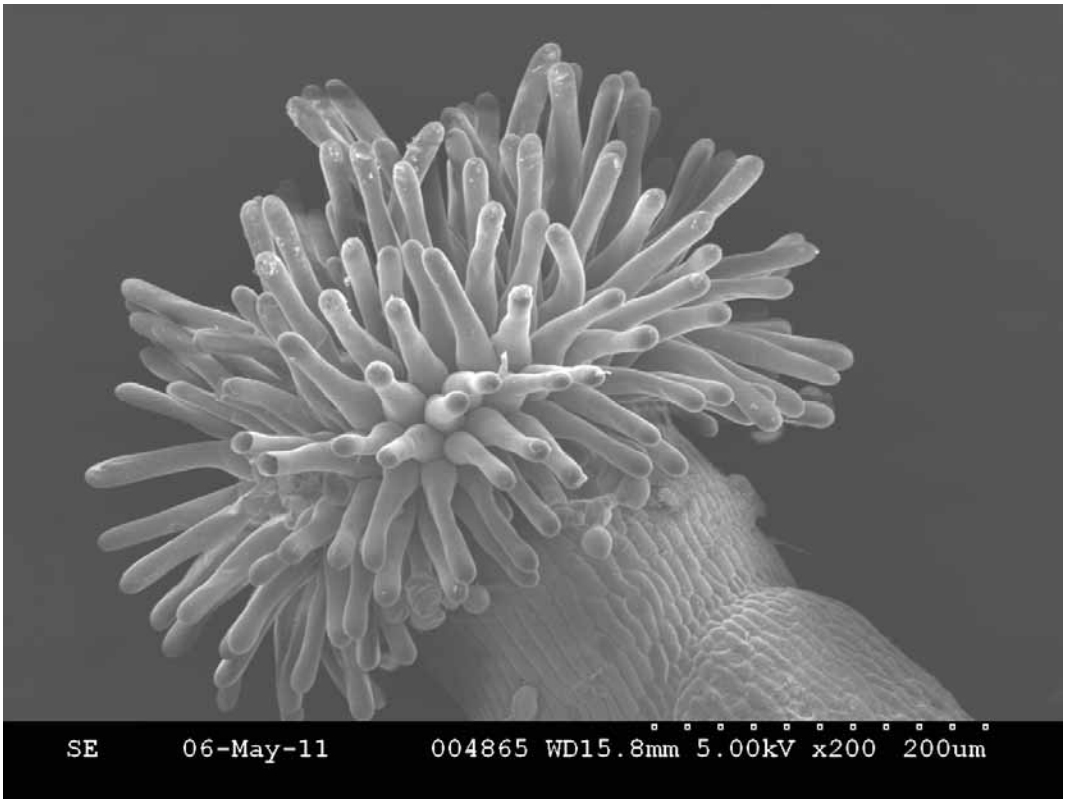
CPD300 auto Program:



Coating:

Gold: 5 nm

Results:



Arabidopsis thaliana flower stigma

Courtesy of Dr. K. Rensing, Application Specialist, Leica Microsystems.

2.1.7 Wrinkled Giant Hyssop Leaf Protocol

Introduction:

Species: Wrinkled Giant Hyssop *(Agastache rugosa)*

Critical point drying of wrinkled giant hyssop leaf with subsequent gold coating and SEM analysis.

Procedure:

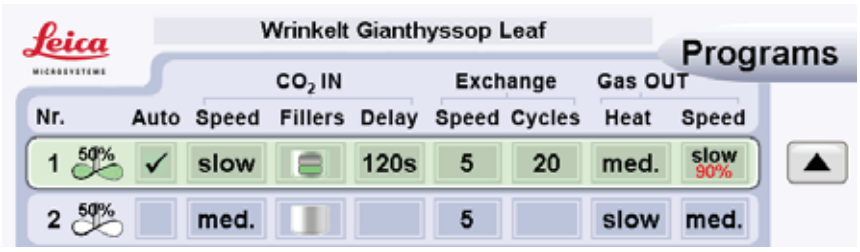
Sample Holder:

Samples were inserted into the 22 mm cover slip holder.

Fixation and Dehydration:

2.5% Glutaraldehyde in 0.1M Sodium Phosphate Buffer, pH 7.2	14 h
0.1M Sodium Phosphate Buffer, pH 7.2	3x 10 min.
Acetone series: 30%, 50%, 70%, 80%, 90%, 95%, 100%	2x 10 min.

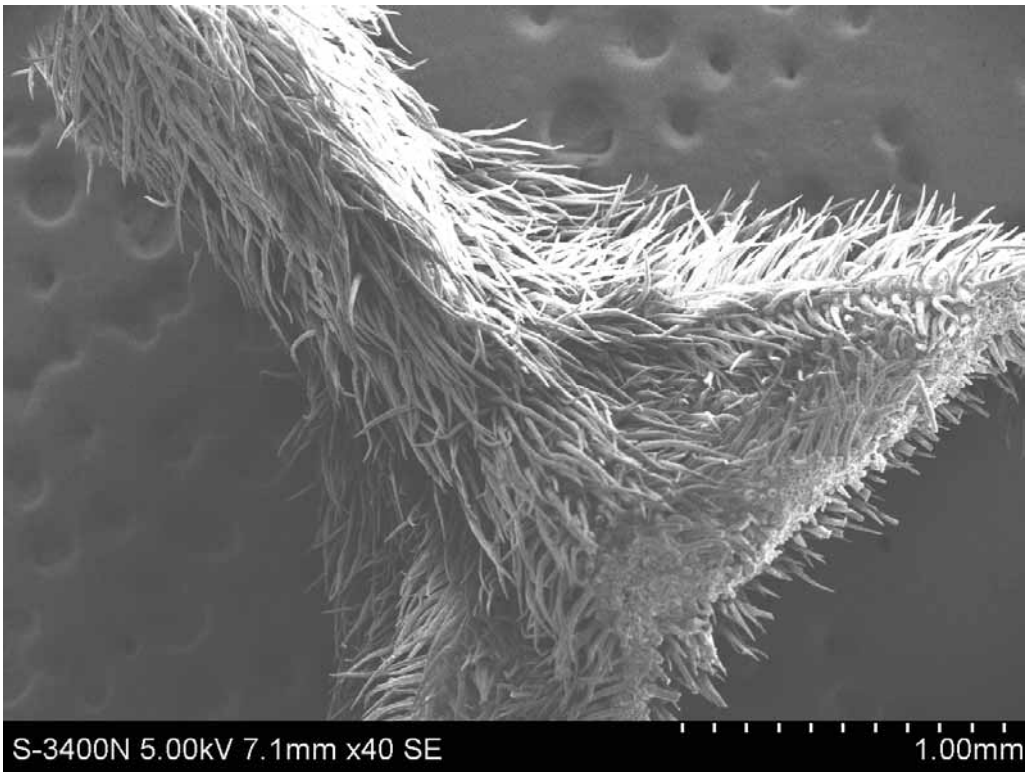
CPD300 auto Program:



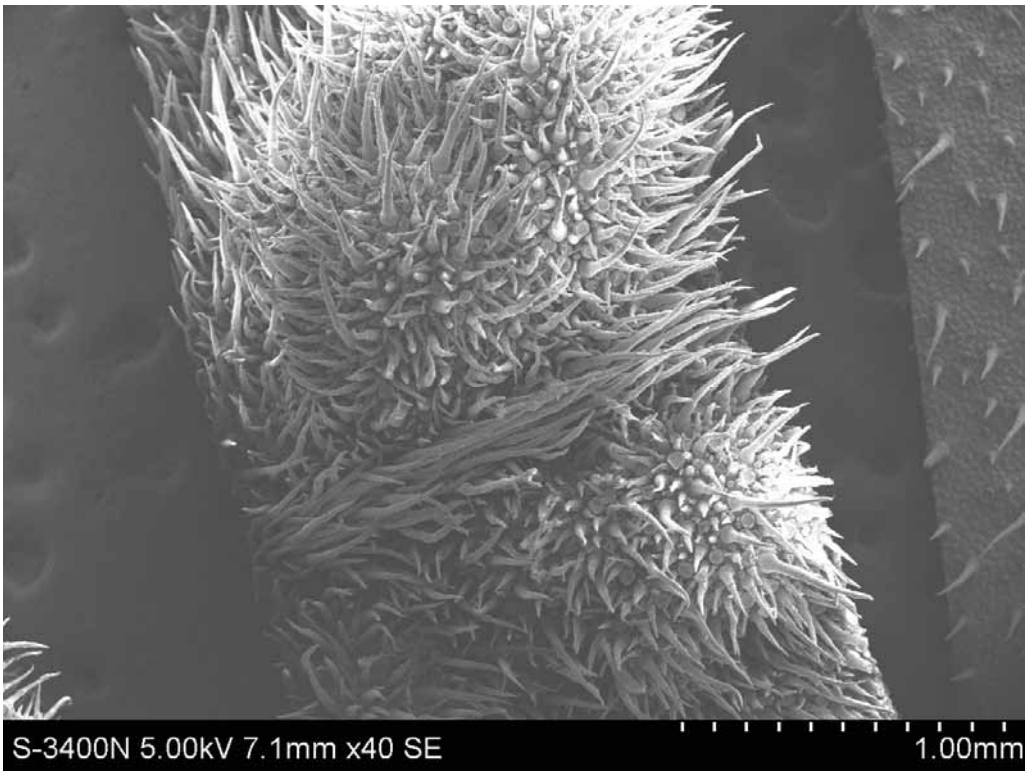
Coating:

Gold: 15-20 nm

Results:



The leaf of Wrinkled Giant Hyssop



Courtesy of Dr. Guo JianSheng, School of Life Sciences and Technology, Tongji University, China.

2.2 Animal / Human Protocols

2.2.1 Human Blood Cells Protocol

Introduction:

Species: Human *(Homo sapiens)*

Critical point drying of human blood with subsequent platinum / palladium coating and SEM analysis.

Procedure:

Sample Holder:

Samples were inserted into the 12 mm cover slip holder.

Preparation

Place 12 mm dia cover slip poly-L-lysine coated in a 12-wells cell culture plate.

Add 1 ml 0.85% NaCl in each well to submerge each cover slip.

Pipette gently 50 µl blood on each glass cover slip leave for 5 min at 25°C.

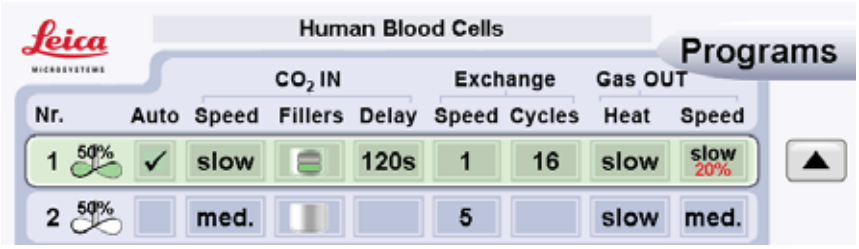
Add 200 µl 0.2 M $CaCl_2$ on top of the blood cells to activate the platelets and leave for 10 min.

Fixation and Dehydration:

Add gently 1 ml of 4% Paraformaldehyde, 0.4% Glutaraldehyde in 0.2 M Sodium Cacodylate Buffer, pH 7.2, on top of the blood cells and leave at least for 10 min. at RT.

Distilled water	3x 10 min.
1% aqueous OsO_4, 4°C	16 h
Distilled water	3x 10 min.
Ethanol series: 30%, 50%, 70%, 80%, 90%, 96%, 100%	1x 10 min.
Acetone series: 30%, 50%, 100%	1x 10 min.

CPD300 auto Program:

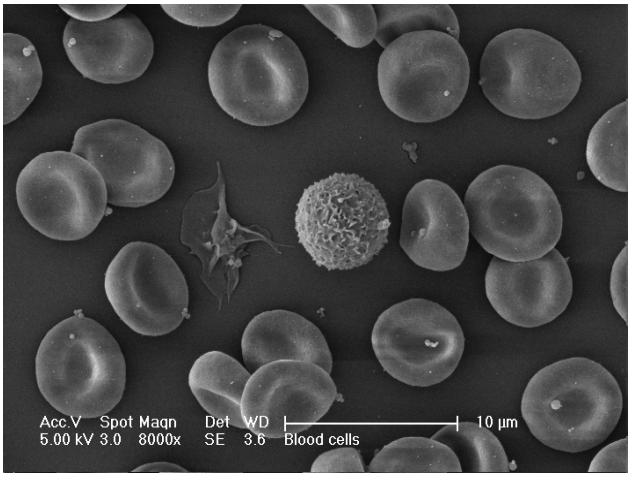


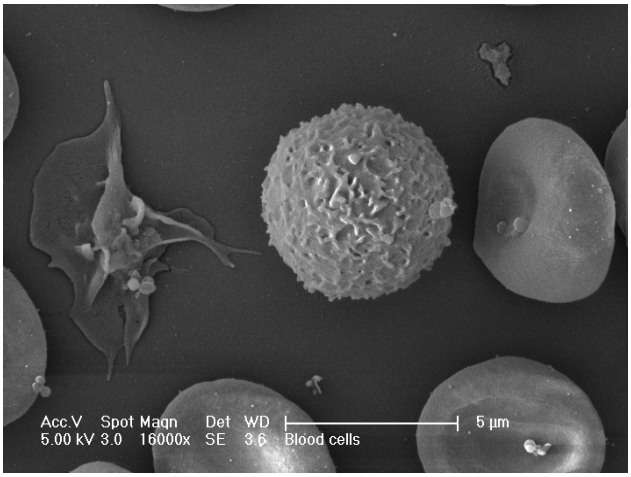
Coating:

Mount the dried samples on stubs containing carbon adhesives.

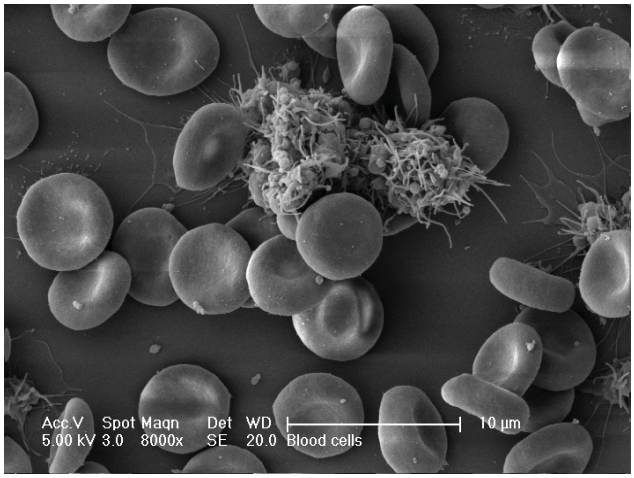
Platinum / Palladium coating: 6 nm

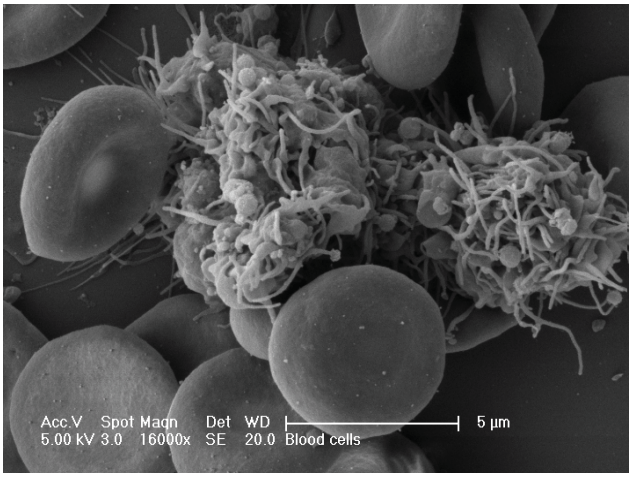
Results:





Human Erythrocytes and Lymphocytes





Human Erythrocytes and Thrombocytes

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

2.2.2 Clawed Frog Nuclear Envelope Protocol

Introduction:

Species: Clawed frog (*Xenopus laevis*)

Critical point drying of nuclear pores from clawed frog oocytes with subsequent chromium coating and SEM analysis.

Procedure:

Sample Holder:

Silicon chips containing the samples were placed into the filter discs and porous pots holder.

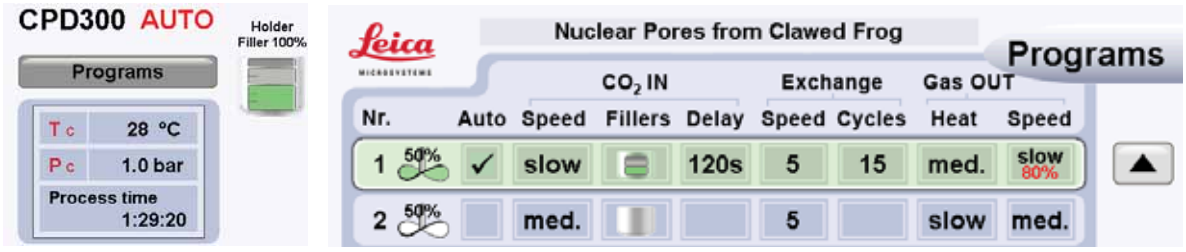
Preparation

Isolated nuclear envelopes were prepared from Xenopus oocytes as described by Goldberg MW, Fiserova J. (2010) Immunogold labelling for scanning electron microscopy. Methods Mol Biol. 657:297-313.

Fixation and Dehydration:

2% Glutaraldehyde, 0.2% Tannic acid, 0.1M Hepes buffer	1x 10 min.
Distilled water	2x 1 min.
0.1% aqueous OsO_4	1x 10 min.
Distilled water	3x 10 min.
Ethanol series: 50%, 70%, 95%	1x 2 min.
Ethanol series: 100%	2x 2 min.

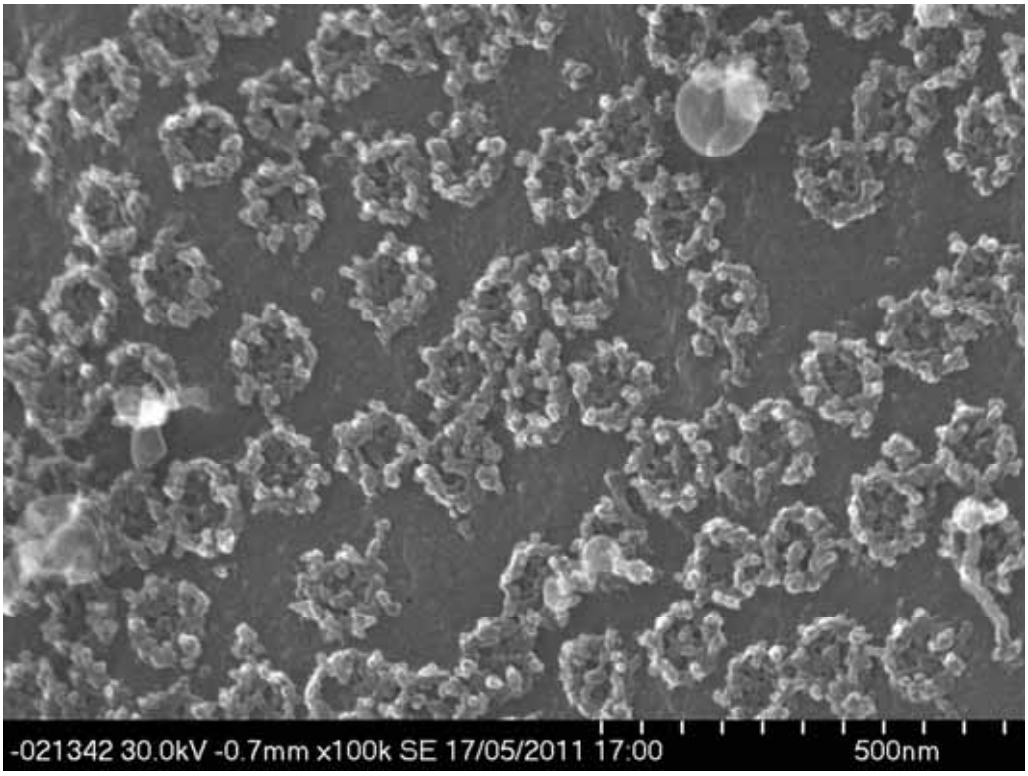
CPD300 auto Program:



Coating:

Chromium: 1.5 nm

Results:



Nuclear pores from clawed frog oocytes

Courtesy of Dr. M. Goldberg and C. Richardson, University of Durham, UK.

2.2.3 Nematode E. dianae Protocol

Introduction:

Species: *Eubostrichus dianae*

Critical point drying of nematode *Eubostrichus dianae* to detect the ectosymbiotic bacteria layer with subsequent gold coating and SEM analysis.

Procedure:

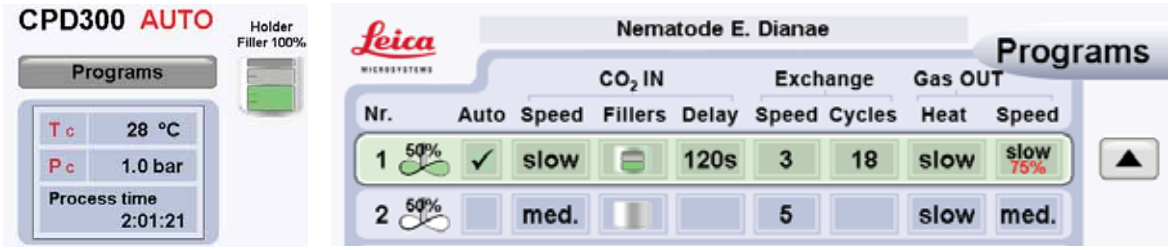
Sample Holder:

Samples were placed into the filter discs and porous pots holder.

Fixation and Dehydration:

2.5% Glutaraldehyde in 0.1M Cacodylate Buffer	2 h
0.1M Cacodylate Buffer	3x 10 min.
1% OsO_4 in 0.1M Cacodylate Buffer	4 -12 h
0.1M Cacodylate Buffer	3x 10 min.
Ethanol series: 30%, 50%, 70%, 80%, 80%, 90%, 90%, 100%, 100%	10 min.
1:1 Mix Ethanol / Acetone	10 min.
100% Acetone	10 min.

CPD300 auto Program:



Coating:

Gold: 10-20 nm

Results:

Eubostrichus with ectosymbiotic bacteria layer

Courtesy of Mag. N. Leisch, University of Vienna, Austria.

2.2.4 Sludge Worm Protocol

Introduction:

Critical point drying of Sludge Worm (*Tubifex tubifex*) with subsequent gold coating and SEM analysis to detect sensory cells on the head of the worm.

Procedure:

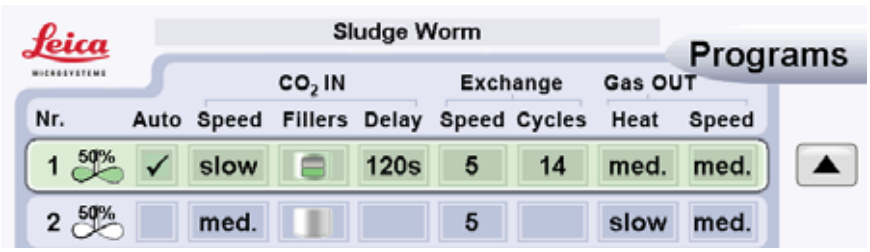
Sample Holder:

Samples were inserted into a filter disc (Pore size: 16 - 40 µm). Filter disc was placed into the cover slip holder 18 mm.

Fixation and Dehydration:

2.5% Glutaraldehyde in 0.1M Sodium Cacodylate Buffer, 2% Sucrose, pH 7.3	1x 2 h
0.1 M Sodium Cacodylate Buffer, 2 % Sucrose, pH 7.3	3x 10 min.
0.1% OsO_4, in 0.1M Sodium Cacodylate Buffer, 2% Sucrose, pH 7.3	1x 1 h
0.1 M Sodium Cacodylate Buffer, 2 % Sucrose, pH 7.3	3x 10 min.
Double distilled water	3x 10 min.
Dimethoxypropane	1x 5 min.
100% Acetone	3x 30 min.

CPD300 auto Program:

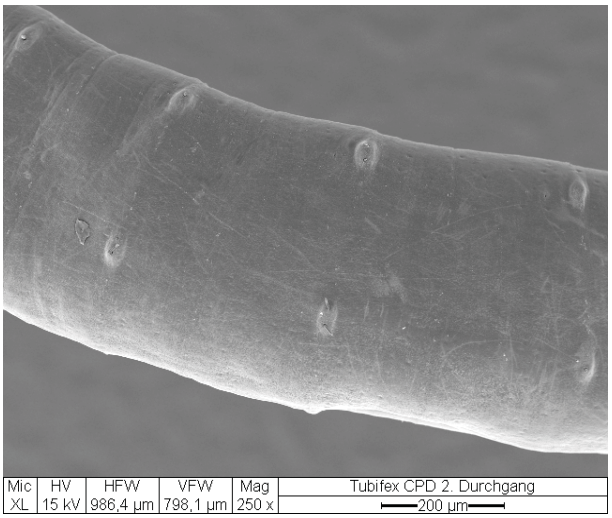


Coating:

Gold: 10-20 nm

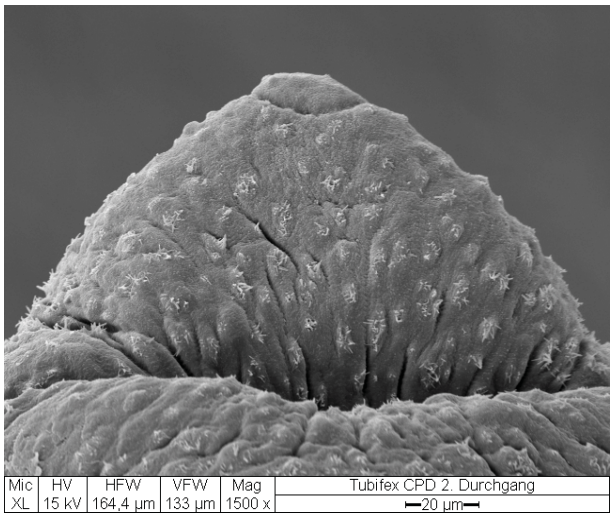
Results:

Mic	HV	HFW	VFW	Mag	Tubifex CPD 2. Durchgang
XL	15 kV	6,8 mm	5,5 mm	36 x	1 mm

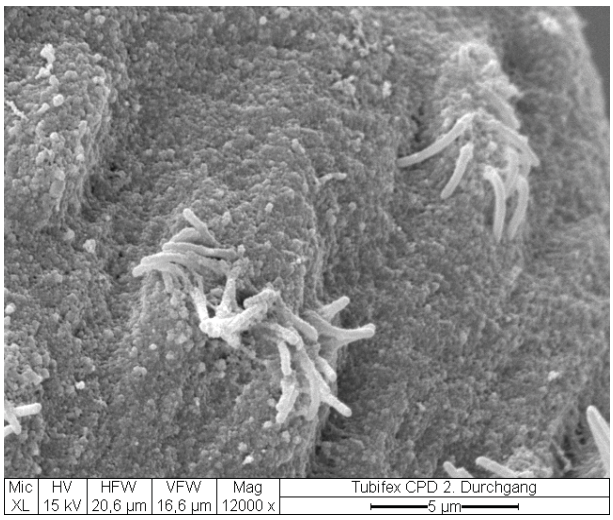


Mic	HV	HFW	VFW	Mag	Tubifex CPD 2. Durchgang
XL	15 kV	986,4 µm	798,1 µm	250 x	200 µm

Sludge Worm



Mic	HV	HFW	VFW	Mag	Tubifex CPD 2. Durchgang
XL	15 kV	164,4 µm	133 µm	1500 x	20 µm



Mic	HV	HFW	VFW	Mag	Tubifex CPD 2. Durchgang
XL	15 kV	20,6 µm	16,6 µm	12000 x	5 µm

Sensoric cells on Sludge Worm's head

Courtesy of Mag. Dr. Gruber, University of Vienna, Austria.

2.2.5 Water Flea Protocol

Introduction:

Critical point drying of Water flea with subsequent gold coating and SEM-Analysis to detect fine surface structures.

Procedure:

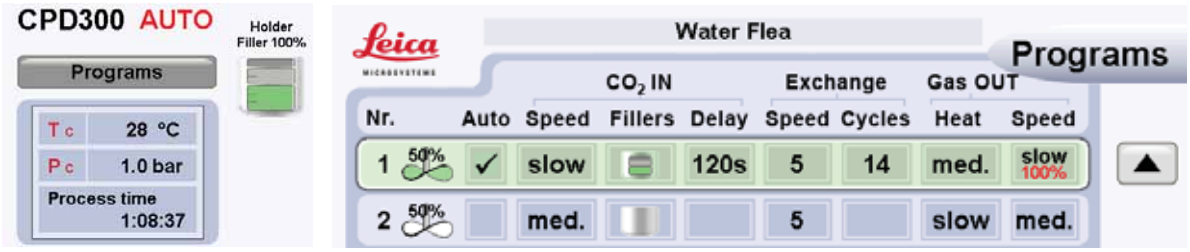
Fixation and Dehydration:

2.5% Glutaraldehyde in 0.1M Sodium Cacodylate Buffer, 2% Sucrose, pH 7.3	1x 18 h
0.1 M Sodium Cacodylate Buffer, 2 % Sucrose, pH 7.3	3x 10 min.
0.1% OsO_4, in 0.1M Sodium Cacodylate Buffer, 2% Sucrose, pH 7.3	1x 1 h
0.1 M Sodium Cacodylate Buffer, 2 % Sucrose, pH 7.3	3x 10 min.
Ethanol 30%, 50%, 70%, 80%, 90%, 96%, 100%	2x 10 min.
100% Acetone, 1% Dimethoxypropane	2x 30 min.

Sample Holder:

Sample was inserted into a filter disc (Pore size: 16 - 40 µm). Filter disc was places into the cover slip holder 18 mm.

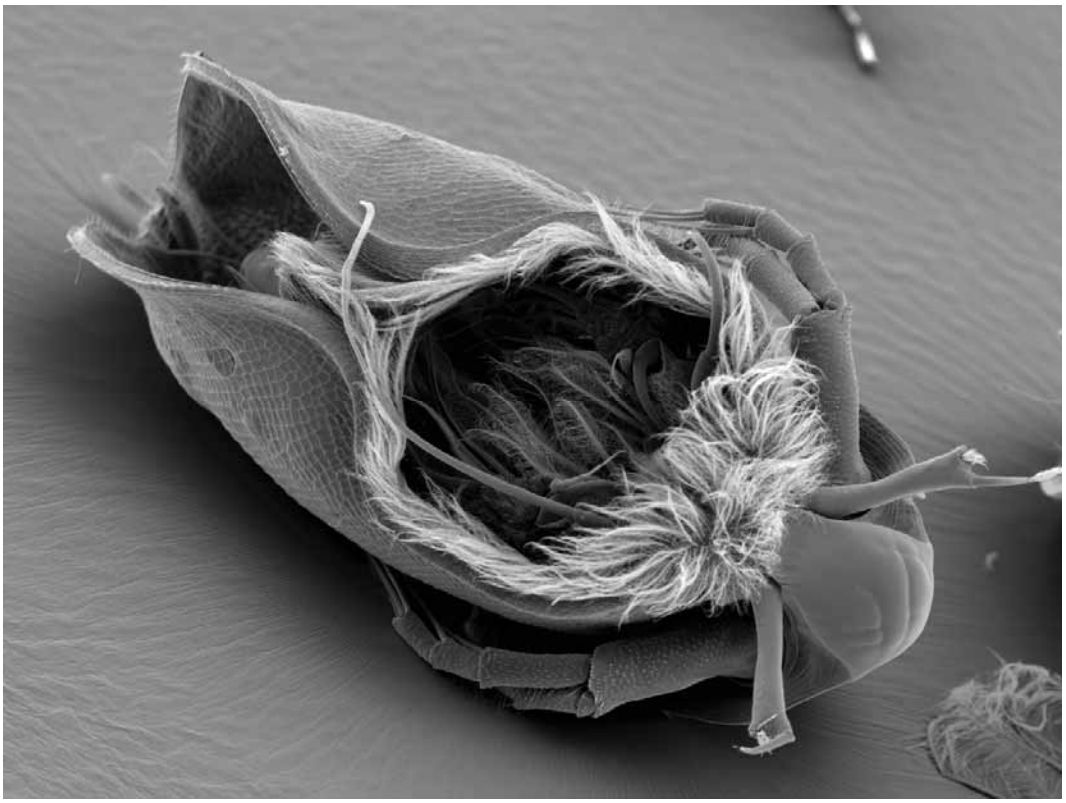
CPD300 auto Program:



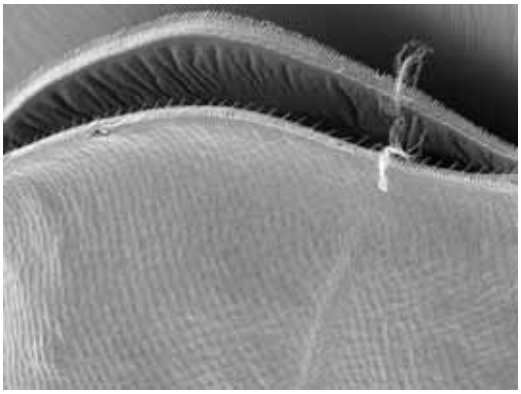
Coating:

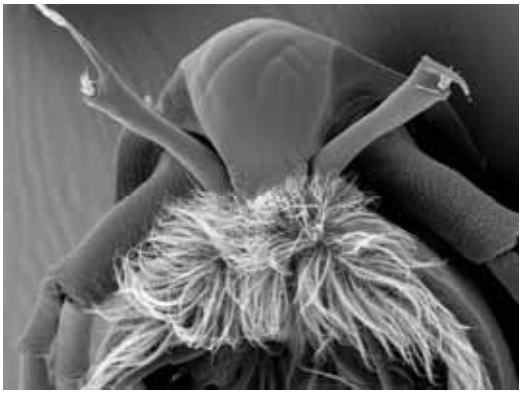
Gold: 10-20 nm

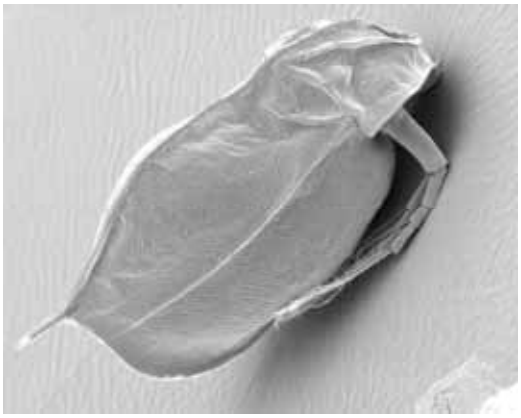
Results:



Water flea







Courtesy of Mag. Dr. Gruber, University of Vienna, Austria.

2.3 Microorganisms Protocols

2.3.1 Bacteria Protocol

Introduction:

Species: *Escherichia coli*

Critical point drying of *E. coli* with subsequent platinum / palladium coating and SEM analysis.

Procedure:

Sample Holder:

Sample were inserted into a filter disc (Pore size: 16 - 40 µm) and placed into the filter discs and porous pots holder.

Cultivation

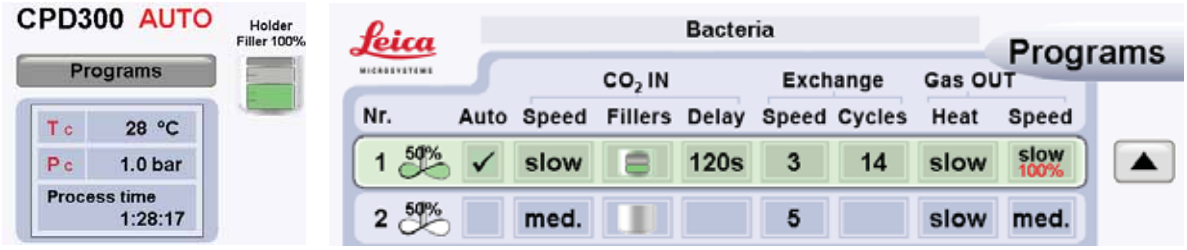
Cultivate fungi and bacteria on agar containing growth medium for 3 days.

Selected parts of the colonies of bacteria

Fixation and Dehydration:

3% Glutaraldehyde in PBS, pH 7.3 at 4°C	16 h
Distilled water	3x 10 min.
1% aqueous OsO_4, at 4°C	16 h
Distilled water	3x 10 min.
Ethanol series: 30%, 50%, 70%, 80%, 90%, 96%, 100% at 25°C	1x 10 min.
Acetone series: 30%, 50%, 100%	1x 10 min.

CPD300 auto Program:

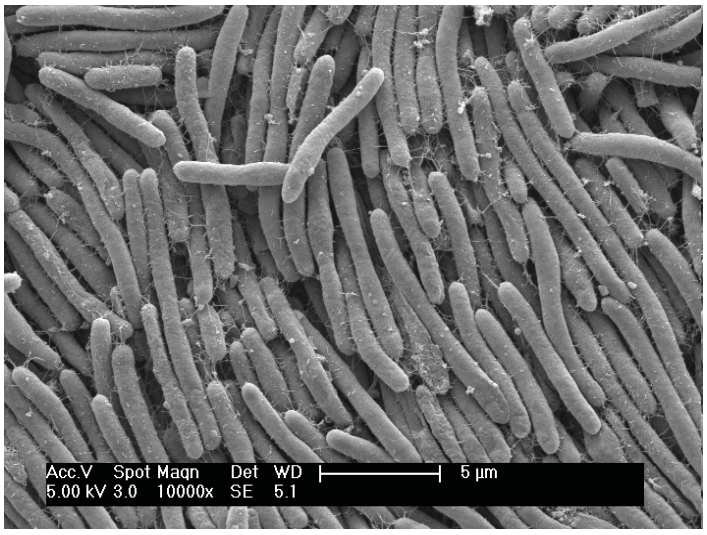


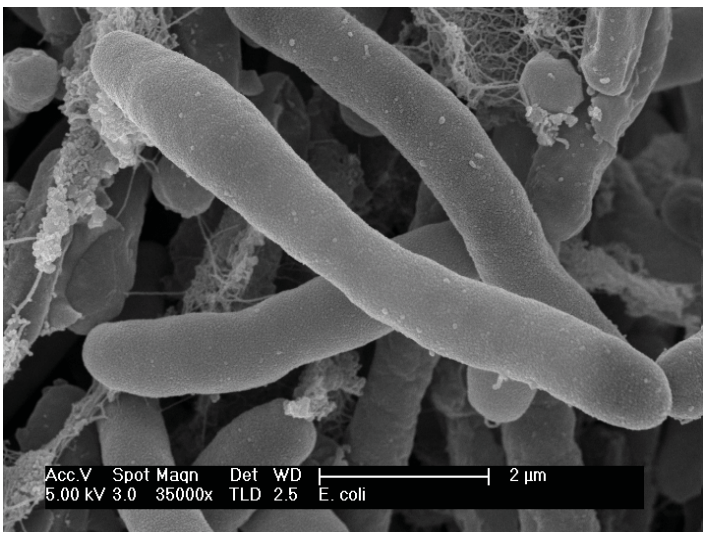
Coating:

Mount the dried samples on stubs containing carbon adhesives.

Platinum / Palladium coating: 6 nm.

Results:





E. coli

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

2.3.2 Black Mold Protocol

Introduction:

Species: Black mould *(Aspergilus niger)*

Critical point drying of Black mould with subsequent platinum / palladium coating and SEM analysis to detect conidiospores.

Procedure:

Sample Holder:

Sample were inserted into a filter disc (Pore size: 16 - 40 µm) and placed into the filter discs and porous pots holder.

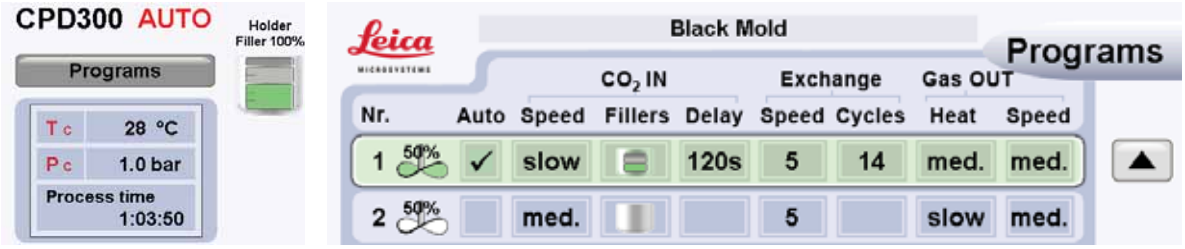
Cultivation

Cultivate fungi on agar containing growth medium for 3 days.

Fixation and Dehydration:

3% Glutaraldehyde in PBS, pH 7.3 at 4°C	18 h
Distilled water	3x 10 min.
1% aqueous OsO_4, 4°C	18 h
Distilled water	3x 10 min.
Ethanol series: 30%, 50%, 70%, 80%, 90%, 96%, 100% at 25°C	1x 10 min.
1% DMP in Acetone series: 30%, 50%, 100%	3x 30 min.

CPD300 auto Program:

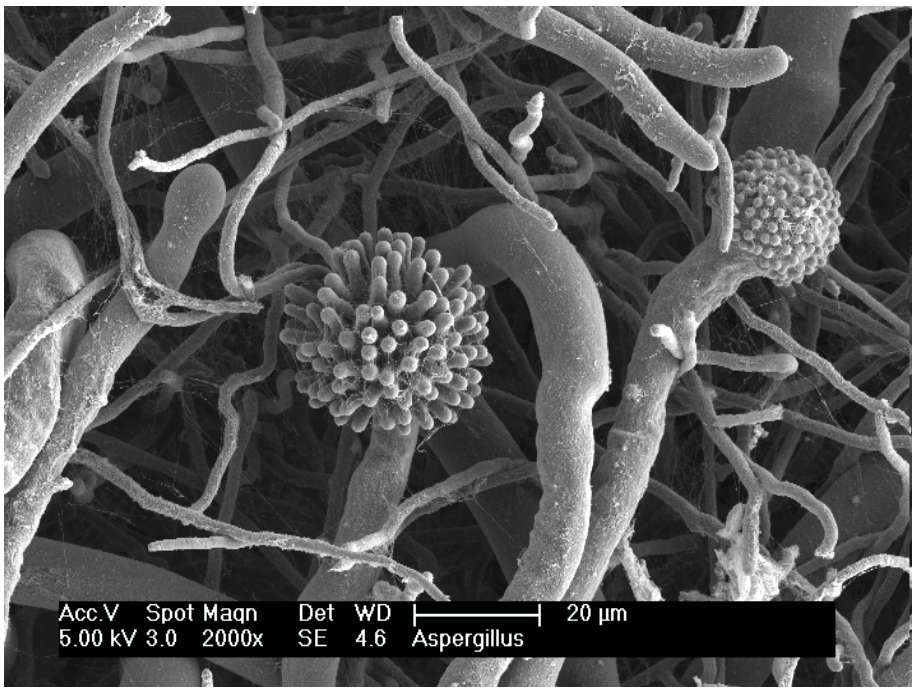


Coating:

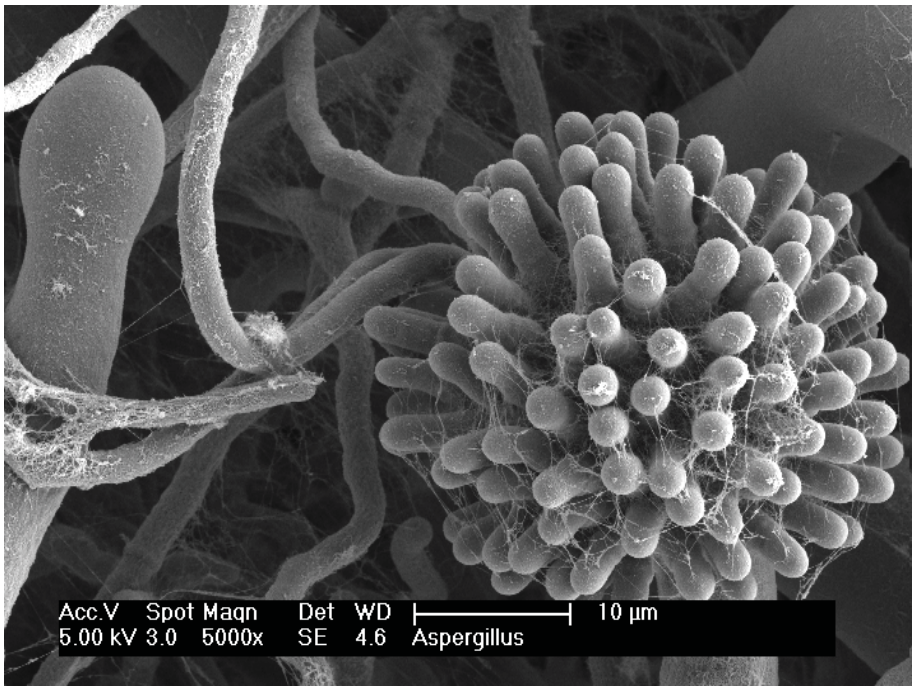
Mount the dried samples on stubs containing carbon adhesives.

Platinum / Palladium coating: 6 nm.

Results:



Black mould conidiospores



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

3. Useful Hints and Tips

3.1 Optimal Working Conditions

CO_2 bottle temperature: 18 – 25 C° (52 – 61 bar)

Relative humidity: 5 – 90%

3.2 CO_2-Bottle Temperature / Pressure Function

For correct filling of the pressure chamber with CO_2 a temperature difference of 4 °C minimum and a pressure difference of 5 bar is essential. Therefore, the pressure chamber has always to be minimum 4 °C cooler than the CO_2-Bottle (see list bellow). You can find the adjustment of pressure chamber temperature under “settings” (see operating manual).
The factory preset cooling temperature of the pressure chamber is 15°C. If the CO_2 does not fill the chamber within a certain time, “Timeout CO_2-IN” shows in the yellow box. If the poral filter is clean and the bottle is not empty the reason for the warning is the CO_2 temperature bottle which is cooler than the chamber temperature. This means, due to the low temperature difference, the pressure of the CO_2 in the bottle is not sufficient to fill-up the chamber.
The temperature of the bottle can be estimated by measuring the bottle surface with a thermometer. The CO_2 temperature is then about 2 °C cooler than the bottle surface. Decrease the chamber temperature according to the list below and fill again. The green marked values indicate the optimal working temperature and pressure range.

Example: If the bottle surface temperature is 22 °C the estimated CO_2 temperature is 20 °C, the cooling temperature of the chamber should be set to 15 °C.

CO_2-Temperature (°C)	Recommended pressure chamber cooling temperature (°C)
14	9
15	10
16	11
18	**13**
20	**15**
22	**17**
24	**19**
25	**20**
26	21
28	23

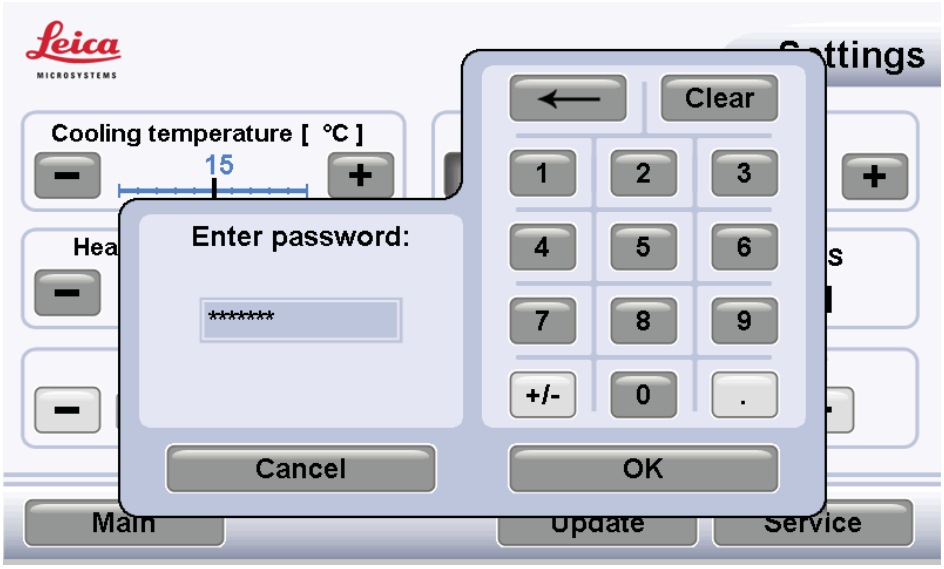
3.3 Adjustments of Pressure Threshold for Bottle Empty Function

The bottle empty function was developed to protect the samples if the CO_2 bottle becomes empty during a run. When the warning occurs, all valves will be closed so that the pressure chamber is sealed and the empty bottle can be exchanged with reduced possibility of sample damage. The threshold for this function has to be adapted to the CO_2 temperature. See list below. Green marked values indicate optimal working temperature and pressure range.

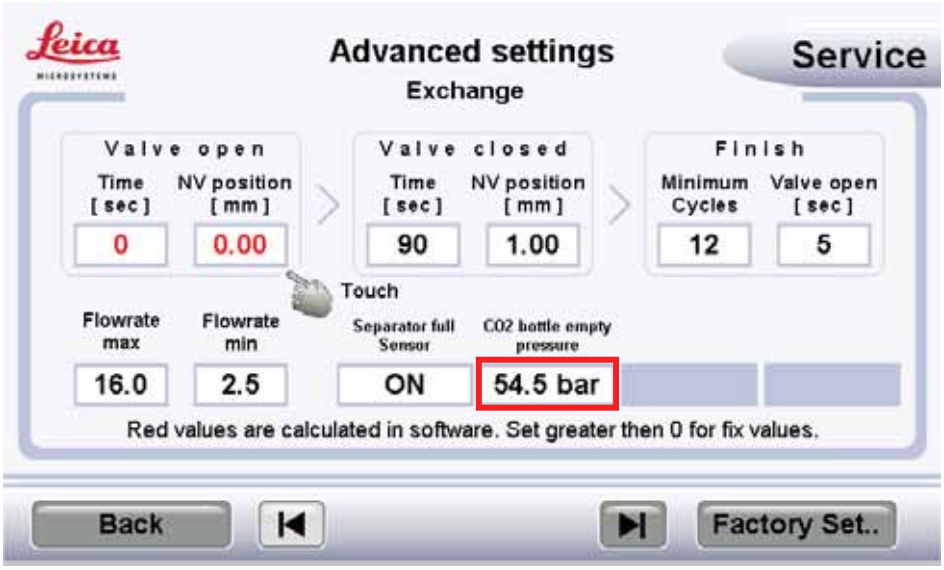
CO_2-Temperature (°C)	Recommended threshold for pressure (bar)	Pressure of full CO_2-Bottle (bar)
14	47	50
15	48	51
16	49	52
18	**52**	**55**
20	**54**	**57**
22	**57**	**60**
24	**60**	**63**
25	**61**	**64**
26	63	66
28	66	69

Adjustments of Pressure Threshold:

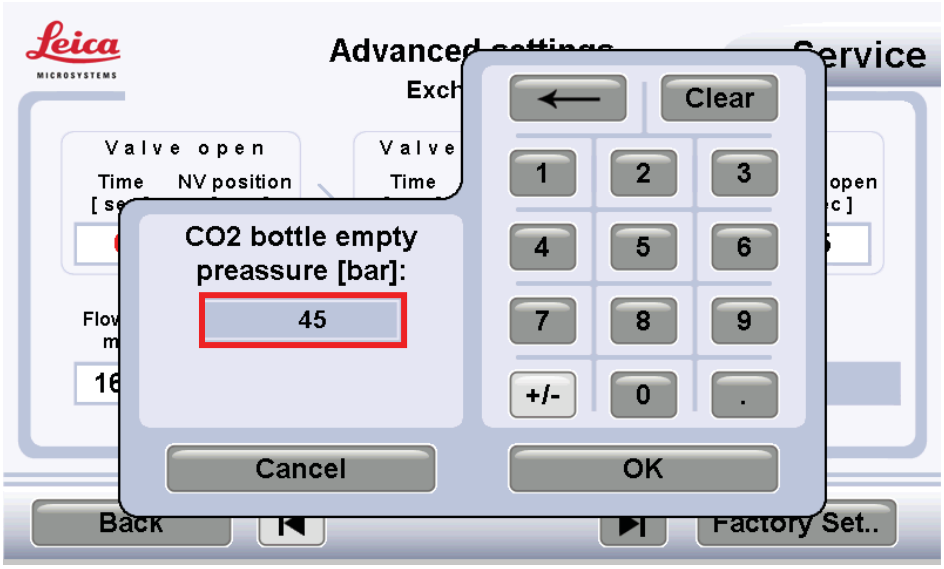
Press Settings, select Service, enter password (see operating manual) and press ok.



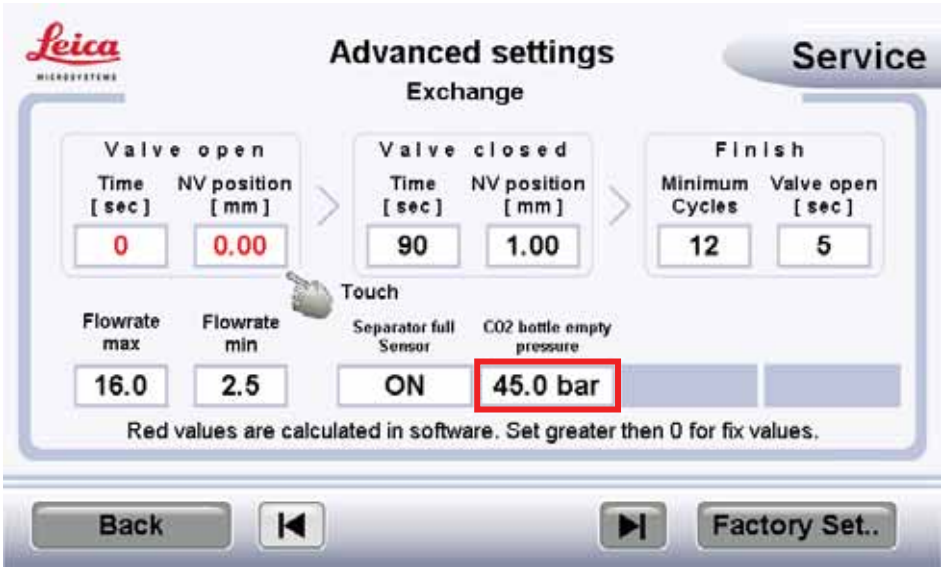
In the advanced settings screen touch the "CO_2 bottle empty pressure threshold" area.



Change CO_2 bottle empty pressure threshold value according to the list on page 45. The CO_2 temperature can be estimated by measuring bottle surface with thermometer. CO_2 temperature is then about 1-2 °C cooler then the bottle surface.



Press „ Back“ to confirm.



3.4 Cleaning

All surfaces can be cleaned with aqueous reagents or 60% ethanol and a clean cloth.

Notes

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