

# Cryo-SEM & Cryo-FIB/SEM Preparation



TECHNIQUES & APPLICATIONS

*featuring the PP3010 Cryo-SEM/Cryo-FIB/SEM Preparation System*

[www.emsdiasum.com](http://www.emsdiasum.com)





# What is...

## Cryogenic Specimen Preparation?

In this instance we are referring to frozen hydrated bulk specimens for Scanning Electron Microscopy (SEM), commonly termed Cryo-SEM. When biological specimens are prepared by alternative methods, such as critical point drying, they may collapse and distort due to the removal of their water content. In addition diffusible elements are often removed or relocated, affecting the validity of subsequent X-ray microanalysis.

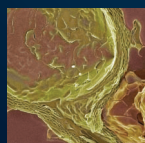
Cryo-SEM offers the best solution to this and in addition allows observation and analysis of liquid, semi-liquid and beam-sensitive specimens, such as emulsions, suspensions and foams. Cryo preparation is increasingly being used with FIB/SEM instruments for a wide range of specimen types, including some materials where low temperature milling conditions are desirable.

For biological and other “wet” specimens rapid freezing is essential in order to reduce morphological distortion, a key consideration for structural observation.

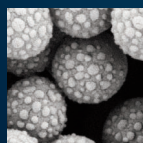
The aim of fast freezing is to reduce the size of ice crystals within specimens by reaching as quickly as possible the point at which recrystallization takes place (for pure water this is in the order of -130°C) and maintaining the specimen below this temperature during transfer, preparation and observation.

For larger specimens commonly used in SEM and FIB/SEM rapid freezing is normally done by plunging into liquid nitrogen in its ‘slushy’ form at -210°C. This is the standard method supplied with the PP3010, but it is also fitted with an “advanced specimen handling” system which allows specimens that have been frozen by alternative (faster) freezing methods to be manipulated and loaded under liquid nitrogen and then transferred under vacuum into the PP3010 for subsequent processing and observation.

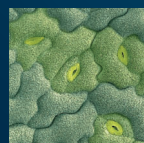
### Cover Photos:



Hand Cream



Latex



Soya Bean Leaf

## Techniques and Applications Cryo-SEM – the advantages

The Scanning Electron Microscopist is faced with the inescapable fact that liquid is a fundamental part of practically all lifesciences – and many materials – specimens. Since water occupies up to 98% of some animal and plant tissues it represents a most formidable specimen problem to most Scanning Electron Microscopists.

Cryo-SEM is a quick, reliable and effective way to overcome these not inconsiderable SEM preparation problems. Additionally the technique is widely used for observing ‘difficult’ samples, such as those with greater beam sensitivity and of an unstable nature. An important application, often overlooked, is the ability to use cryo-SEM to study dynamic processes (industrial or otherwise) by using a series of time resolved samples.

Naturally the advent of various “higher pressure” modes, such as VP, LV and ESEM has allowed such samples examined in SEM without resorting to freezing or drying methods. However, cryo-SEM is still by far the most effective method of preventing sample water loss, which will in fact occur at any vacuum level – even with Peltier stages fitted to the SEM and the careful addition of water vapor in the SEM chamber. Cryo-SEM also a number of additional advantages, including the ability to fracture and selectively remove surface water (ice) by controlled specimen sublimation.

### Why choose cryo-SEM?

The limitations of conventional ‘wet processing’ include:

- Shrinkage and distortion
- Extraction of soluble materials
- Relocation of highly diffusible elements
- Mechanical damage (fragile specimens can be damaged during conventional processing)
- Slow (24 hours or longer)
- Toxic reagents are required (fixatives, buffers etc)

### Advantages of cryo-SEM:

- Specimen viewed in fully hydrated state
- Soluble materials are retained
- Less relocation of highly diffusible elements
- Little or no mechanical damage
- Time lapse experiments and evaluating industrial processes at timed intervals
- Usually no exposure to toxic reagents
- Rapid process
- High resolution capability (compared to low-vacuum techniques)
- Extra information obtained by low-temperature fracturing (compared with conventional and low-vacuum methods)
- Good for liquid, semi-liquids and beam sensitive specimens
- Ability to selectively etch (sublimate to reveal information)
- Ability to ‘rework’ specimen (eg re-fracture and coat)

## Learn how to do it...

Our EMS Microscopy Academy offers a Cryo SEM Workshop for individuals who are new to the field of cryo SEM, desire a technical refresher to maintain current skills, or just want to see and learn all of the possibilities of the technology.

This course will cover the process of rapid freezing, fracturing, coating, and imaging of a variety of samples.

### Main Curriculum

- Theory and overview of cryo SEM
- Mounting and adhering
- Freezing, loading and fracturing
- In situ coating
- Operation of SEM
- Cryo face-off Leica UC 7 Crion
- Specific techniques of Cryo SEM imaging



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Microscopy  
Academy

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# Techniques and Applications

## A summary of the cryo-SEM preparation technique

Cryo preparation techniques for scanning electron microscopy (SEM) have become essential for the observation of wet or 'beam sensitive' specimens. Using such techniques removes the need for conventional preparation techniques, such as critical point drying or freeze-drying, and allows observation of the specimen in its 'natural' hydrated state.

The specimen is rapidly cooled and transferred under vacuum to the cold stage of the preparation chamber, which is mounted onto the SEM chamber. The preparation chamber is pumped either with a rotary pump (PP2000) or by a specially designed turbomolecular pumping system (PP2000T). The specimen can be fractured, sublimated ("etched") to reveal greater detail, and coated with metal by sputtering or with carbon by thermal evaporation.

Finally, the specimen can be moved under vacuum into the SEM chamber where it is easily located on a cold stage specifically tailored to the SEM. At all stages of the procedure the specimen is maintained at a 'safe' temperature of typically lower than -140°C.

### Typical applications

Biological sciences including botany, mycology, zoology, biotechnology and biomedical – plus economically import agricultural sciences.

More recently cryo-SEM is becoming an essential tool for pharmaceutical, cosmetics and healthcare industries, where it is used in basic applied research and for routine QA of many products, such as creams, cosmetics and drug delivery systems.

Cryo-SEM has long been a standard preparation method in the food industry. Of interest are multi-

phase products, such as ice cream, confectionery and dairy products.

Botanical: Cryo-SEM is the perfect method for highly hydrated botanical material.

### Some specimen mounting techniques for cryo-SEM

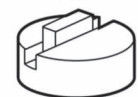
#### Surface mounting

This technique is used for leaf specimens etc. Roughen stub surface with fine emery paper. Specimen is laid on top of mounting media.



#### Edge mounting

This technique is used for edge observation and fracture. Roughen surface of stub with fine emery paper. Specimen is placed on its edge in a machined slot and secured with mounting media.



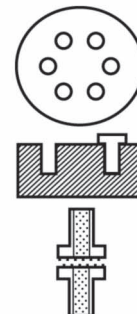
#### Film emulsion mounting

This technique is useful when a small specimen would be obscured by the Tissue-Tek mounting media, or when specimens need to be recovered. Specimens need to be slightly damp to use this method (good for nematode worms).

The specimen is laid on surface so that its dampness slightly dissolves the film emulsion allowing the specimen to adhere to the film surface. Exposed unused film with the emulsion side uppermost is secured to the stub with mounting media. It may be useful to scrape off the protective coating of the film emulsion first to assist conductivity.

### Rivet mounting

For liquids and for when specimens need to be frozen off the stub to achieve fast freezing rates. The rivet is placed in the hole and filled with liquid prior to freezing. If the specimen needs to be frozen away from the stub, two liquid-filled rivets are held together and then frozen prior to transfer onto the stub.



### Alternative rivet mounting method

Pipette liquid sample into hole in sample stub

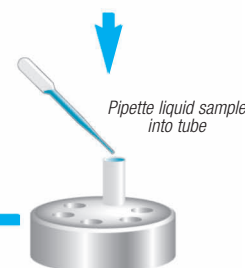


Place metal rivet or small piece of plastic tubing on top of hole (containing liquid sample).

Note: Small drop of "Super Glue" can be used to hold tube to stub.

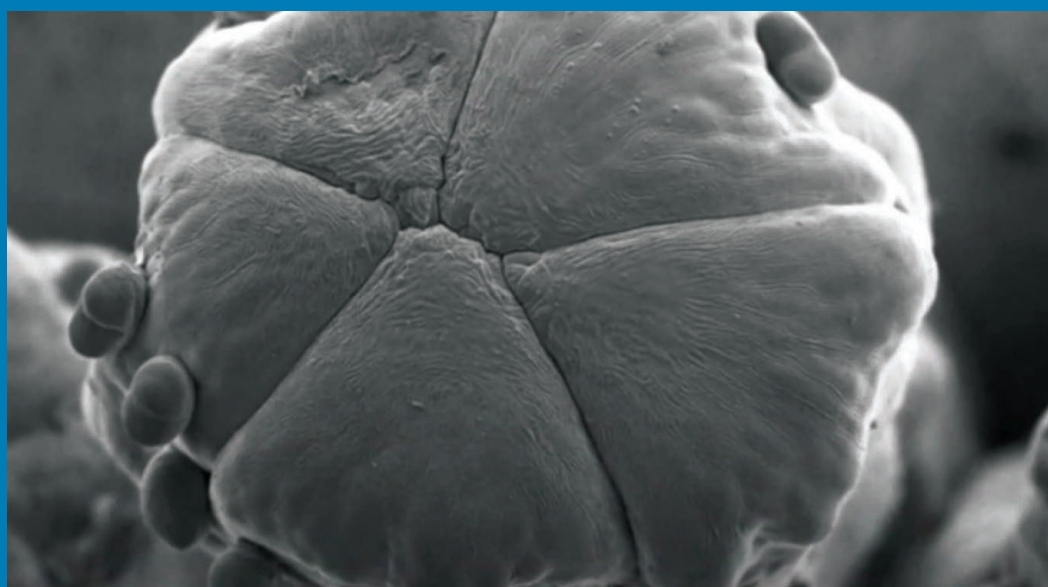


Freeze & transfer onto preparation chamber cold stage



Pipette liquid sample into tube

Result: clean surface fracture







## OVERVIEW

The PP3010 is a highly automated, easy-to-use, column-mounted, gas-cooled cryo preparation system suitable for most makes and models of SEM, FE-SEM and FIB/SEM.

The PP3010 has all the facilities needed to rapidly freeze, process and transfer specimens. The cryo preparation chamber is turbomolecular pumped and includes tools for cold fracturing, controlled automatic sublimation and sputter coating. After processing, the specimen is transferred from the cryo preparation chamber onto a highly stable SEM cold stage for observation. Cold trapping in the cryo preparation chamber and SEM chamber ensures the whole process is frost-free. Specimen process times are typically between five and ten minutes.

## PP3010 Cryo-SEM/Cryo-FIB/SEM Preparation System

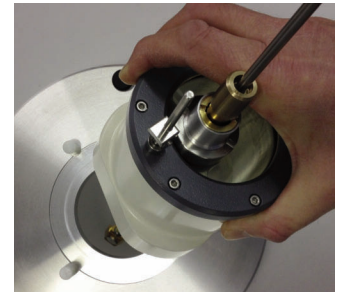
### KEY FEATURES

- High resolution performance on SEM, FE-SEM and FIB/SEMs
- Totally gas cooled, including cryo preparation chamber – no boiling nitrogen on the SEM
- Efficient cooling (down to at least  $-190^{\circ}\text{C}$ )
- 24 hours plus run times on one fill of LN<sub>2</sub> are typical – allowing unattended overnight operation (at typical operating temperatures)
- Large recipe driven touch-screen interface
- Automated sublimation, coating and system start up
- Superb specimen visibility (including preparation chamber CCD camera)
- Fully compatible with SEM beam deceleration/stage bias modes up to 5kV
- Off column cooling and pumping systems – minimum mass on the SEM
- On-screen data logging and diagnostics
- Pumped storage of the cryo transfer device
- Prepdek™ workstation – self contained work area, extra bench space not required
- Cryo workflow options
- Specialist support and three-year warranty

### PRODUCT FEATURES

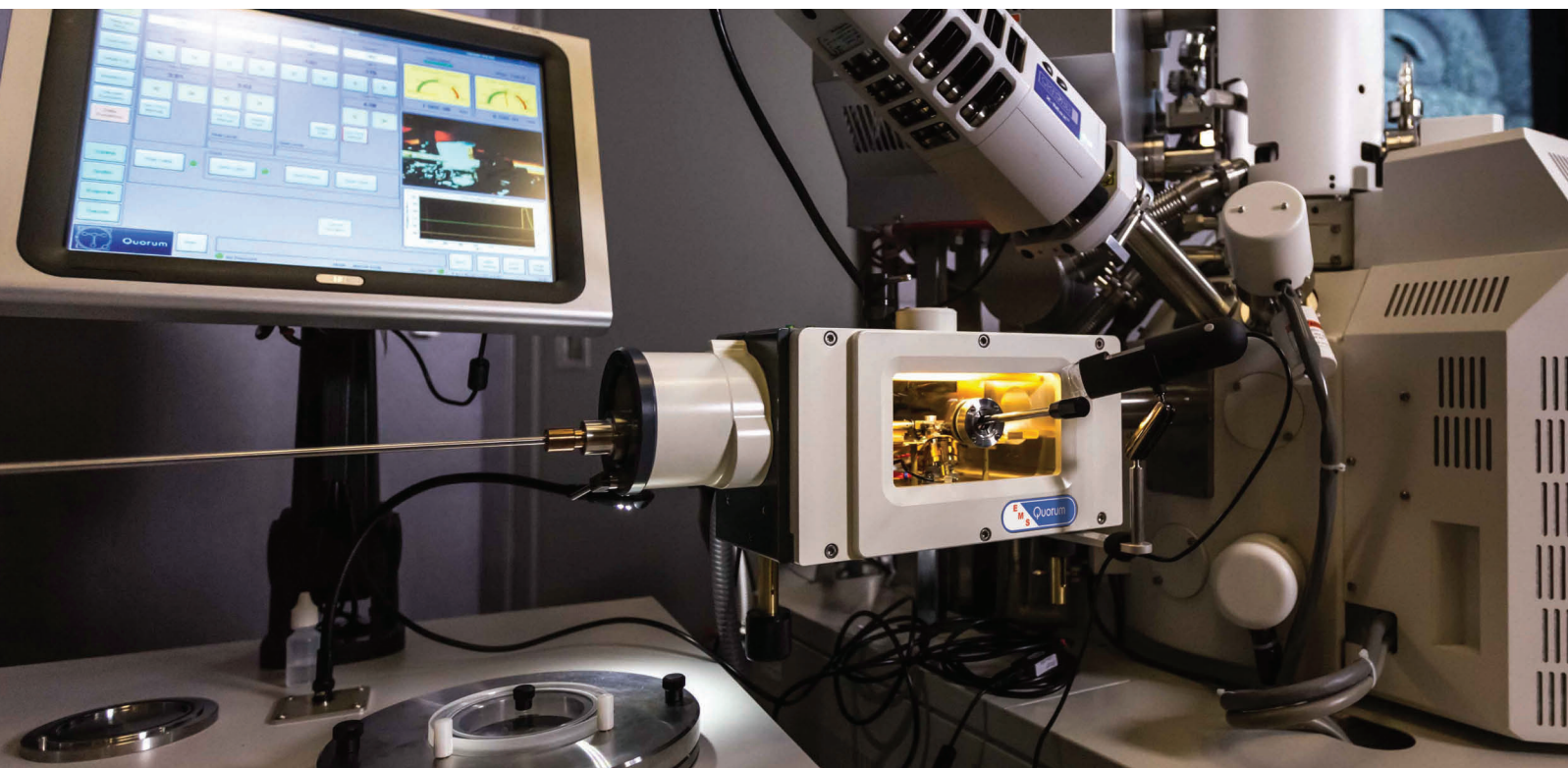
#### Mounting, Freezing and Transferring Specimens — easy with the Prepdek™ Workstation

The PP3010 Prepdek™ workstation is fitted with a slushy nitrogen freezing station, connected to the pumping system. Rapid freezing reduces ice crystal damage and results in improved specimen preservation. For handling pre-frozen material the Prepdek™ freezing system allows specimens that have been frozen by alternative freezing methods (or stored field specimens) to be manipulated — in or just above liquid nitrogen — and then transferred under vacuum into the PP3010 preparation chamber for subsequent processing and observation.



*Slushy nitrogen freezing station*

Additionally the TEM Prep Slusher and Glove Box Interface/Airlock options allow workflow amongst a range of other platforms, including cryo-TEM, cryoultramicroscope, XPS and glove box.



### III PP3010 Cryo-SEM/Cryo-FIB/SEM Preparation System (continued)

#### Cryo Transfer Device – Including Vacuum Storage

The vacuum transfer device is compact (fits easily into one hand), reliably vacuum-tight and has a bayonet connection to the specimen shuttle to ensure rapid pick up and transfer.

Set into the Prepdek™ work surface is a pumped storage tube for the cryo transfer device (see Prepdek™ workstation section below).

#### Specimen Stubs, Shuttles

The PP3010 is supplied with universal 10 mm specimen stubs with surface slots, holes and a flat area – useful for most specimen types. Blank and slotted stubs are also included. In addition a range of optional holders is available, including shuttles for large specimens and top-loading holders for high pressure freezing, TEM Autogridstm (for cryo-FIB/SEM applications) and clamping shuttles for hard specimens.

#### Cryo Preparation Chamber

The cryo preparation chamber is connected directly to the microscope and includes a highly efficient nitrogen gas cold stage, extensive cold trapping and facilities to fracture, sublimate and sputter coat specimens. The chamber is fitted with two fully integrated and interlocked gate valves. The outer load-lock valve includes a pumped airlock which accepts the cryo transfer device – the inner SEM valve ensures rapid high-vacuum to high-vacuum specimen exchange.

#### Highly Efficient Gas Cooled Stage and Cold Traps

At the heart of the cryo preparation chamber is a nitrogen gas cooled specimen stage. The stage has a dovetail fitting to accept a cryo shuttle and can be precisely controlled over a temperature range from 100°C to -190°C or lower. Large gas cooled cold traps located above, below and behind the specimen stage ensure clean, high vacuum conditions in the chamber.

Both the cold stage and cold traps are cooled with the fully integrated CHE3010 off-column cooling system (see below), which at normal operating temperatures give typical hold times of at up to 24 hours between fills (provided the nitrogen gas is dry).

#### High Visibility – Plus CCD Camera

There is superb visibility into the preparation chamber. In addition to the large front window (75 x 150 mm), there are two top viewing ports. The chamber is lit by three LEDs and a CCD camera allows the specimen cold stage area to be viewed on the control screen and the images saved.

An optional stereo microscope can be fitted to the cryo preparation chamber.

#### Cold Fracturing

Twin fracturing tools manipulators (actively cooled) are available and allow a range of specimen types to be cold fractured.

The PP3010 is fitted as standard with a front mounted fracturing and manipulation device. The ball-jointed mount offers flexible movement of the blade which can be used both as a surface pick (probe) and a fracturing knife.

An optional micrometer-advanced fracturing tool with rigid blade is available, in addition to the standard front-mounted tool.

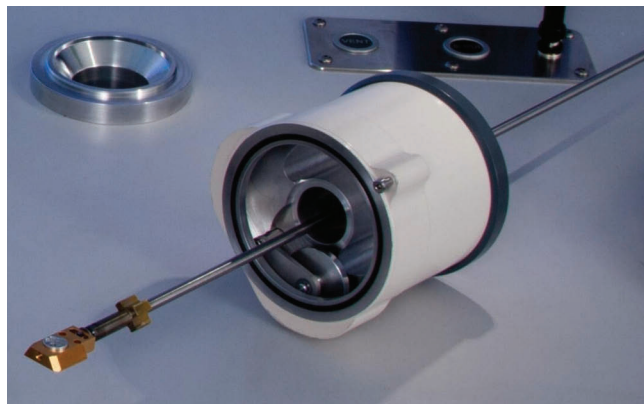
Fractured fragments are captured in the large cold trap located below the specimen stage.

#### Automatic Sublimation and Sputtering

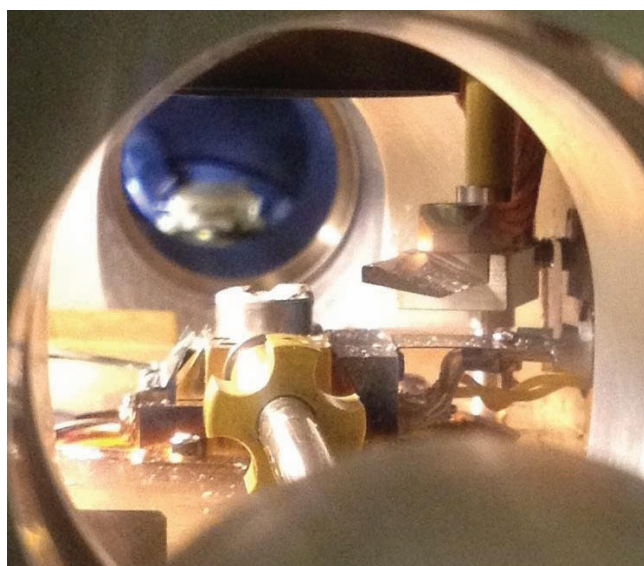
Sublimation temperatures and times can be preset and stored for easy retrieval. The process is fully automatic and graphically displayed on the control screen, showing the actual versus the predicted temperature curves.

The high resolution sputter coater is based on the market leading series of bench top coaters. The coating system will give fine grain films essential for FE-SEM applications. A platinum target is fitted as standard – optional metals include gold, gold/palladium, chromium and iridium. An optional fully integrated carbon fiber evaporation head can also be fitted.

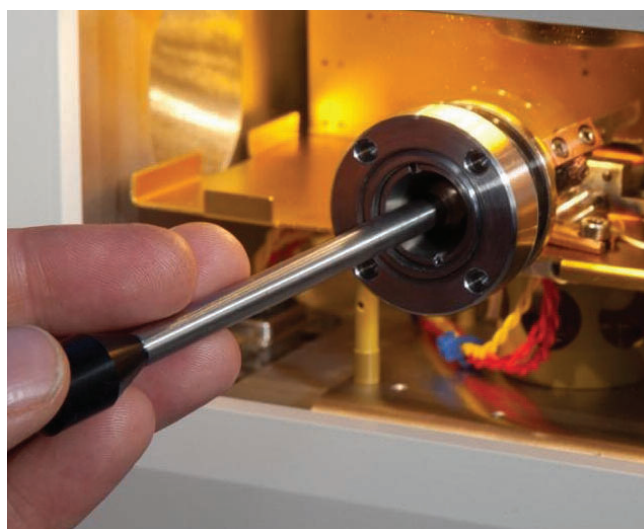
An optional terminating film thickness monitor is available.



*Specimen transfer device*

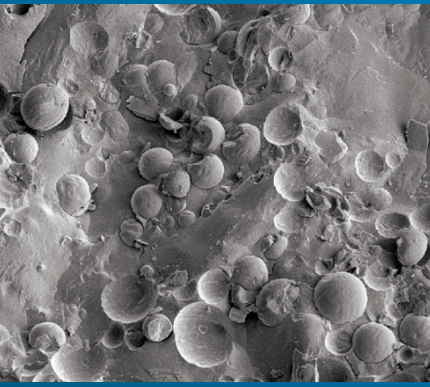


*View during specimen transfer*



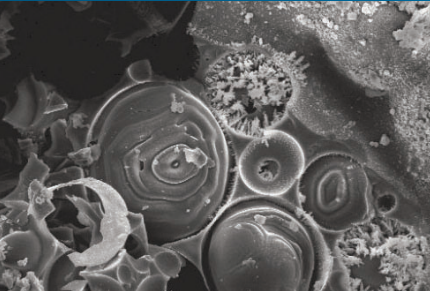
*Front-mounted fracturing and specimen manipulation tool*





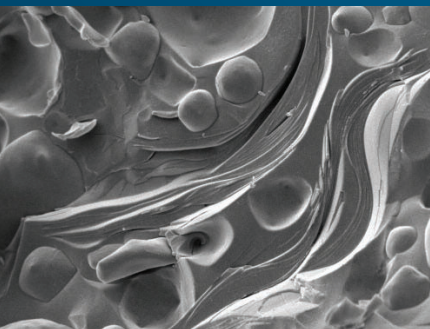
**Mayonnaise**

Image courtesy of FEI Company.



**Shaving Cream**

Fractured at -140°C, sublimated at -90°C for 3 minutes and coated with 5nm of platinum.



**Face Cream**

Anti-aging face cream. Specimen rapidly frozen in slushy nitrogen, fractured at -140°C and sputter coated with 5nm of platinum

### III PP3010 Cryo-SEM/Cryo-FIB/SEM Preparation System (continued)

#### Turbomolecular Pumping – High Vacuum Performance

The preparation chamber is pumped by a remotely-positioned 70 L/s turbomolecular pumping system. Typical preparation chamber vacuums when cold are in the region of  $10^{-7}$  mbar or better. Positioning the turbomolecular pump away from the SEM ensures total elimination of mechanical vibration and has the advantage of significantly reducing the total cryo system mass connected to the SEM. A vacuum buffer tank (remotely located in the Prepdek™) is automatically pumped when required. The pumping system is connected to the preparation chamber by flexible stainless-steel bellows, which allows flexible positioning of the pumping system.

A 5 m<sup>3</sup>/hr rotary vacuum pump is required to "back" the turbomolecular pump and for slushing and rough pumping operations. The rotary pump can be located up to five meters from the system, allowing remote location if required. Dry pumping alternatives are available – see Ordering Information.

#### Prepdek™ Workstation

The Prepdek™ workstation has been designed to allow specimen mounting, freezing (plus pre-frozen specimen manipulation) and transfer device storage on one ergonomically designed work surface. The control electronics are mounted in a sealed but accessible cabinet beneath the Prepdek™.

Set into the work surface is a pumped storage tube which allows the cryo transfer device to be stored under vacuum conditions when not in use.

#### Panel PC Touch Screen User Interface

The PP3010 is controlled using a large touch screen panel PC, mounted on the Prepdek™ workstation. User-defined 'recipes' can be entered and stored for instant future access. The screen can be set to suit operator preferences; for example, vacuum measurement can be displayed in millibar, Pascal or Torr.

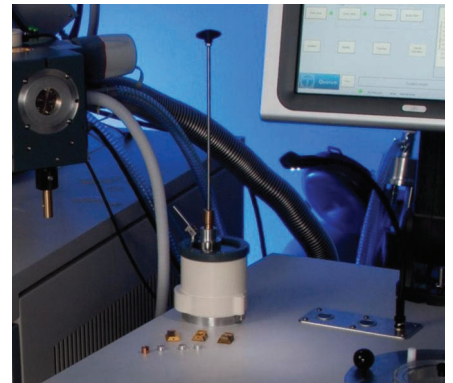
Many of the key steps in the specimen preparation process are automated (system set up, gas flow control, sublimation and sputter coating).

#### SEM Cold Stage, Cold Trap and Cooling System

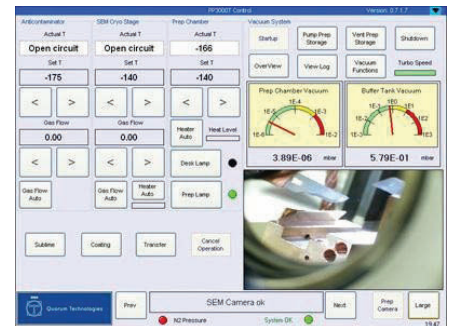
A highly stable, thermally isolated, nitrogen gas-cooled stage attaches to the SEM stage. The SEM stage and cold trap are cooled by separate cold gas circuits – both capable of reaching temperatures of -190°C or lower. This configuration allows the operator to select stage and cold trap temperatures that are optimized for specific specimens. For example, for some non-biological materials, it is useful to hold the specimen at very low temperatures – for example, a cold stage temperature of -175°C. This is possible with the PP3010, as cold trap temperatures of -190°C or lower can be selected, but not possible with conduction cooled



Remotely mounted turbomolecular pumping system

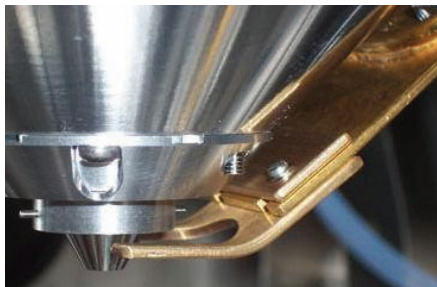


When not in use, the cryo transfer device can be stored under vacuum in the pumped storage tube, located on the Prepdek™ work surface



Typical screen view during operation (with camera image minimized)

### III PP3010 Cryo-SEM/Cryo-FIB/SEM Preparation System (continued)



*Gas-cooled SEM cold trap (temperatures down to -190°C). Tailor-made to suit each SEM*

systems. The SEM cold stage has a temperature range of down to -190°C and a temperature stability of < 0.5°C.

#### Compatibility with SEM Stage Bias Mode

The PP3010 cold stage is fully compatible with SEM stage bias/beam deceleration modes of up to 5kV.

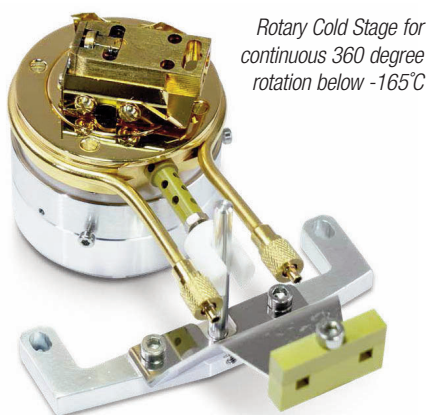
#### CHE3010 Off-Column Cooling

The CHE3010 is a fully integrated, remotely mounted cooling system which comes as standard with every PP3010. The CHE3010 is used to cool the SEM stage, SEM cold trap and cryo preparation chamber cold stage and cold traps and will typically reach temperatures down to -90°C or lower.

The CHE3010 is remotely positioned (typically on the floor behind the microscope) and at normal operating temperatures can run for up to 24 hours between fills. This greatly simplifies the cryo process (no more checking on dewar status and topping off), but also allows overnight, unattended operation – particularly useful for some automated FIB/SEM "slice and view" protocols.

#### Single Port Interface to the SEM or FIB/SEM

Where SEM geometry allows, both the cryo preparation chamber and the SEM cooling system can be fitted to a single chamber port (the minimum port diameter is 38 mm). This gives a tidy installation and frees up a valuable chamber port.



*Rotary Cold Stage for continuous 360 degree rotation below -165°C*

## OPTIONS AND ACCESSORIES

### Rapid Warming Tower for the CHE3010

This device is meant to facilitate a rapid warming of the CHE3010 (Cryo-Heat Exchanger) associated with the PP3010 Cryo-SEM preparation system. The Rapid Warming Tower in conjunction with a 1 liter/min Nitrogen gas flow rate dramatically reduces the lag time before the SEM or Prep chamber components return to room temperature so venting can occur. A process that normally takes hours can be completed in minutes, allowing maintenance or reconfiguring the SEM back to room temperature operation. Accepts many common, locally obtained hair dryers. (Hair Dryer not included).



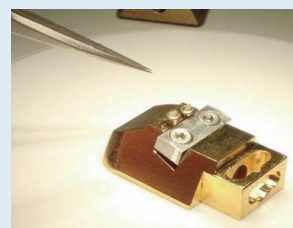
*Rapid Warming Tower*

### Specimen shuttles and stubs

The PP3010 is supplied with a selection of holders, and a range of additional specimen shuttles and stubs is also available. (See Ordering Information for details).

### Carbon Evaporation and Film Thickness Monitor

A carbon evaporation attachment and a terminating film thickness monitor can be fitted. Both are fully integrated (no external control boxes required).



*Specimen shuttle*

### Pressurized LN<sub>2</sub> Dewar

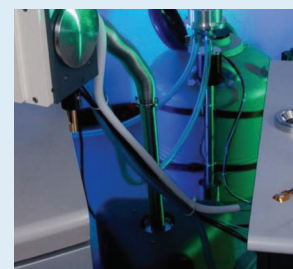
The PP7450/60L is a highly recommended option that generates dry nitrogen gas used for cooling the SEM cold stage & cold trap and cryo preparation chamber and cold shields. In addition, LN<sub>2</sub> can be decanted for slushing (freezing). During normal operation the PP7450/60L will generate dry nitrogen gas for up to eight days usage.

If the PP7450/60L is not included, appropriate, locally sourced, nitrogen gas cylinders can be used. It is important to ensure that it has low moisture content – if in doubt, please contact us.

### Cryoflow Work Options

#### Glove Box Valve/Airlock Interface

The airlock is connected to a glove box using a generic NW fitting and for most applications requires a suitable pumping system (rotary pump or turbomolecular plus diaphragm pumping system).



*Gas cooling dewar and turbo pump*

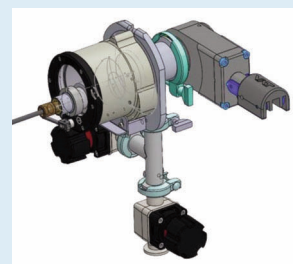
The airlock is designed to accept the PP3010 vacuum transfer device.

For full details of this and other accessories please see Ordering Information.

#### TEM Prep Slusher Option

Offers an easy, convenient way of transporting pre-frozen specimens to and from the PP3010.

- Conveniently locates into the Prepdek™ workstation of the PP3010
- Freely transportable, e.g. between high pressure freezer, cryoultramicrotome and the PP3010
- Ideal for loading/unloading TEM grids and grid holders
- Tilting holder for cryo shuttles (holders). Allows the easy transfer of specimens from external freezers (e.g. high pressure, jet, slam etc)
- Option for PP3010 and previous PP3000T and PP2000/PP2000T models

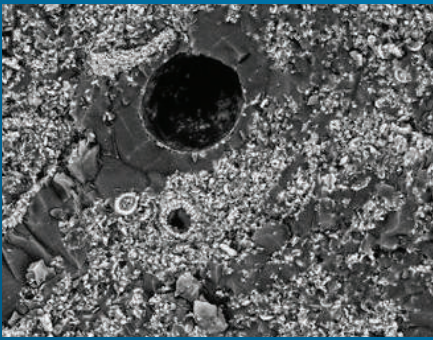


*Glove box valve/airlock interface*

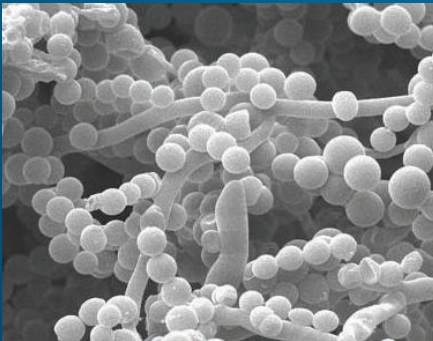


*Cryo preparation chamber with cryo transfer device fitted*

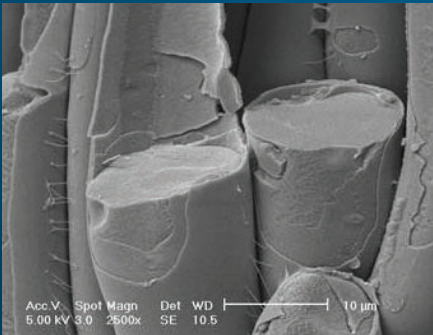




Cross-section of oil/water/rock.



Cryo prepared image of blue stilton cheese (Penicillium roqueforti).



Cross-section through plant palisade cells.



Cross-section image through sunscreen

## SPECIFICATIONS

### Cryo Preparation Chamber (column-mounted)

	Standard?
Gas cooled preparation chamber with a twenty-four hour run time between fills	Yes
Two integral gate valves (loading and SEM) with appropriate electrical interlocks	Yes
Variable temperature gas-cooled specimen stage	Yes
Large cold shield above, below, behind the cold stage	Yes
Robust micrometer-fed fracturing knife (actively cooled)	Option
Side-mounted surface knife/probe (actively cooled). A range of scalpel blades can be fitted to suit different specimen requirements	Yes
Automatic sublimation (controlled and viewed on the touch screen)	Yes
Fully automatic, high resolution sputter coater with platinum (Pt) target. (Other targets, including gold (Au), gold/palladium (Au/Pd), chromium (Cr) and iridium (Ir), are available as options.) Sputtering controlled and viewed on the user touch screen	Yes
Carbon fiber evaporation head and power supply	Option
Large front viewing window (150 x 78mm) plus top viewing ports	Yes
Preparation chamber camera (CCD)	Yes
Vacuum transfer device	Yes
Chamber illumination — three LEDs	Yes

### Pumping System and Controls

Remotely-mounted turbomolecular pumping system (70L/s). Includes: vacuum buffer tank, vacuum valves and stainless-steel bellows connection to the preparation chamber.	Yes
Typical preparation chamber vacuum when cold: 10 <sup>-7</sup> mbar	
Single 50L/m rotary pump required	Order separately

### SEM Cooling Dewar, SEM Cold Stage and Cold Trap (anticontaminator)

Gas-cooled nitrogen cold stage assembly (-190°C). Temperature stability of >0.5°C	Yes
Separate gas-cooling circuits for SEM stage and SEM anti-contaminator	Yes
21L capacity, off-column cooling dewar with run time between fills of up to 24 hours	Yes
SEM CCD camera-fitted when space allows	Yes
LED lighting (interlocked)	Yes

### System Control and Specimen Handling

Control via a color user touch screen monitor (15") mounted on the Prepdek™	Yes
<ul style="list-style-type: none"> <li>• Multi-ability user interface screen</li> <li>• Quick, easy overview of system status</li> <li>• User-definable "recipes" can be stored</li> <li>• Quick access to videos outlining preparation techniques and system maintenance</li> <li>• Fully automatic sputtering</li> <li>• Automatic sublimation</li> <li>• Quick, easy overview of system status</li> <li>• CCD camera image of preparation chamber</li> </ul>	

Twin liquid nitrogen slushing and specimen handling system — ideal for handling pre-frozen specimens. Mounted on the Prepdek™

System electronics stored in a ventilated, sealed unit under the Prepdek™

Specimen shuttles (x2). E7449-9 multi-specimen stubs (pack of 10) and E7402 blank aluminium (Al) stubs (pack of 10). Other shuttles and stubs available — see Ordering Information

### Specimen Shuttles and Stubs (Others available — see Ordering Information)

<ul style="list-style-type: none"> <li>• (2) Specimen shuttles (to hold 10 mm diameter cryo stubs)</li> <li>• Blank 10 mm stubs — pack of 10</li> <li>• Multi-stubs 7 mm high (with holes and slots) — pack of 5</li> <li>• Multi-stubs 5 mm high (with holes and slots) — pack of 5</li> <li>• Dovetail holder shuttle</li> <li>• Brass rivets for fracturing liquids — pack of 100</li> <li>• Copper (Cu) stub with 3 mm x 3 mm slot — pack of 5</li> <li>• Copper (Cu) stub with 1 mm x 3 mm slot — pack of 5</li> </ul>	Yes
---	-----

### Installation and Training

Installation and training at the customer site

### Support and Other Information

Comprehensive start-up kit with key spares	Yes
Three-year warranty	Yes
SEM column interfaces and SEM stage adaptor (tailored to each microscope)	Yes

### Some Options and Accessories (see Ordering Information for full list)

Terminating film thickness monitor (FTM)	Option
Self-pressurizing LN <sub>2</sub> dewar and regulator (for storage and venting)	Option
Carbon fiber evaporation head	Option
Wide range of specimen holders and specimen stubs	Option

Contact EMS



### III PP3010 Cryo-SEM/Cryo-FIB/SEM Preparation System (continued)

#### ORDERING INFORMATION

For a full quotation, including on-site installation and customer training, please contact us.

<b>PP3010</b>	<b>Turbo Pumped Cryo System for SEM, FE-SEM and FIB/SEM.</b> Includes 13297 Sircal gas dryer. For a full detailed description please contact us	each
<b>Pumping</b>		
PP3010 has an integrated turbomolecular pump, but also requires a 13034 rotary pump or 20063 oil-free scroll pump		
<b>13034</b>	Pfeiffer DUO-6 5m <sup>3</sup> /hr rotary vacuum pump with oil mist filter	each
<b>20063</b>	Edward NXDS6i oil-free scroll pump (replaces 13034 rotary pump)	each
<b>Shipping</b>		
<b>13064</b>	Wooden crate and packing for export	each
<b>Installation and Training</b>		
<b>PP3010-I</b>	Installation and training - Four day on site installation and customer training	
<b>PP3010-T</b>	Engineer costs - Travel from PA to customer site, including local hotels	
<b>PP3010 Options and Accessories</b>		
<b>PP7450</b>	60 liter pressurized liquid nitrogen dewar. Used for generating dry nitrogen gas required to cooling the SEM stage and cold trap plus preparation chamber cold stage and cold traps. If not ordered, cylinder gas can be used (check purity requirement with EMS)	each
<b>11000</b>	Rotary Cold Stage: standard stage alternative, tilts up to 52°, rotates 360°, maintains desired cryotemperatures	each
<b>10996</b>	Glove Box Interface/Airlock – for vacuum or inert gas transfer	each
<b>10997</b>	TEM Prep Slusher option for transporting pre-frozen specimens to and from the PP3010	each
<b>13297</b>	Sircal nitrogen gas dryer 220-240V	
<b>13298</b>	(100-110V part: 13298). <i>Note: useful if quality of the nitrogen gas source is unknown. Included with PP3010</i>	each
<b>PP7424</b>	Binocular stereo microscope with fitting to the PP3010 cryo preparation chamber. Note: the PP3010 preparation chamber is fitted with a CCD camera as standard. Both PP7424 and CCD can fitted together	each
<b>20996</b>	Carbon fiber evaporation head and power supply, including 100cm of high purity carbon fiber.	each
<b>12147</b>	Terminating film thickness monitor (FTM)	each
<b>12145</b>	Micrometer controlled fracturing device with tooled steel blade.	each
<b>PP3050-RWT</b>	CHE3010 Rapid Warming Tower	each
<b>12340-SPARE</b>	Cryo transfer device (spare) - one included with PP3010	each
<b>Specimen Shuttles (dovetail).</b> Some will accept 10mm dia.		
<b>AL200077B</b>	Standard specimen shuttle with 10m hole for cryo stub. Two included with PP3010	each
<b>12434</b>	Blank specimen shuttle for large specimens. Total area: 290 mm <sup>2</sup>	each
<b>20718</b>	Blank specimen shuttle with extended length for large specimens. Total area: 350 mm <sup>2</sup>	each
<b>20720</b>	Shuttle for large, flat specimens (with light microscope-style stage clips)	each
<b>13524</b>	Shuttle for clamping hard, flat specimens. Suitable for flat specimens (front of shuttle with clamp lever) and cross-fracturing (sprung-loaded vice at rear of shuttle).	each
<b>12013</b>	For clamping, larger flat specimens (handle removed after loading specimen). Useful for loading pre-frozen specimens	each
<b>25276-Q</b>	Shuttle for clamping hard flat specimens (clamps for upright and flat mounting)	each
<b>10245</b>	Cryo shuttle for high pressure freezer ("Balzer") planchette-style holders	each
<b>13054</b>	Shuttle for clamping hard, flat specimens. Suitable for flat specimens (front of shuttle shown with clamp lever) and cross-fracturing (sprung-loaded vice)	each
<b>20529</b>	Top loading specimen holder shuttle (similar to AL200077B but stub clamping mechanism is located on the top – useful for handling pre-frozen specimens mounted on a stub). One included with PP3010	each
<b>13419</b>	Tilt-rotate shuttle. Tilt and rotation can be altered on the preparation chamber cold stage using system knife/probe	each
<b>20530</b>	Top loading, vice style specimen shuttle for directly clamping two 328116510 fracturing rivets	each
<b>12406</b>	TEM grid holder cryo shuttle, including cryo shield, for cryo-FIB/SEM applications. Takes two TEM "Autogrids"	each
<b>12922</b>	Cryo shuttle for FEI Polara™ cartridge	each
<b>13359</b>	STEM shuttle for 3mm TEM grid, includes grid location tool. For STEM detectors mounted below the SEM cold stage	each
<b>Specimen Holder Stubs (10mm dia.)</b> For use with appropriate shuttles		
<b>E7433</b>	10mm diameter specimen stub for freeze fracture rivets (fits into 10246)	each
<b>E7402</b>	Specimen stub, aluminum (10mm dia. X 5mm high) blank, pkt of 10. One packet included with PP3010	each
<b>E7449-5</b>	Multi-purpose specimen stub with slots and holes, 10 mm dia. X 7 mm high (pack of five). One pack included with PP3010	each
<b>11541</b>	1 x multi-purpose specimen stub with slots and holes (10 mm dia. X 5 mm high) blank. Five included with PP3010	each
<b>E7402</b>	Aluminum stubs, 10 mm dia. x 5 mm, high, blank, pkt of 10. One packet included with PP3010	each
<b>E7403</b>	Specimen stub, copper (10mm dia. X 5mm high) blank, pkt of 10.	each
<b>E7405</b>	Specimen stub for clamping thin, flat specimens x 1	each
<b>E7406</b>	Specimen stub, copper with 3mm wide x 3mm deep slot, pkt of 5	each
<b>E7407</b>	Specimen stub, copper with 1mm wide x 3mm deep slot, pkt of 5	each
<b>328116510</b>	Rivets for liquid fracture (pkt 100). One packet included with PP3010	each
<b>Spares Kit</b>		
<b>13061</b>	Two-years consumable and basic spares kit for PP3010	each
<b>Sputtering Targets and Carbon Fiber</b>		
(All targets 24.5 mm in diameter) - all other target types available upon request		
<b>E7400-314A</b>	Gold (Au) sputtering target, 24mm dia. X 0.2mm thick	each
<b>E7400-314B</b>	Gold/Palladium (Au/Pd) sputtering target, 24mm dia. X 0.2mm thick	each
<b>E7400-314C</b>	Platinum (Pt) sputtering target, 24mm dia. X 0.2mm thick. One included with the PP3010	each
<b>E7400-314IR</b>	Iridium (Ir) sputtering target, 24mm dia. X 0.3mm thick	each
<b>E7400-314CR</b>	Chromium (Cr) target 0.3 mm thick	each
<b>91047-1</b>	Carbon fiber cord - standard grade (1M)	each
<b>91047-5</b>	Carbon fiber cord - standard grade (10M)	each



## III Cryo Transfer Systems

Building on the success of the PP3010 cryo-SEM/FIB/SEM preparation system, we are pleased to announce three new related products for ambient and cryo temperature transfer.



PP3004 QuickLok

### PP3004 QuickLok

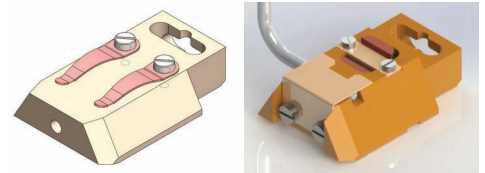
Ambient temperature airlock for SEM, FIB/SEM, beamline and vacuum platforms

#### Quick Overview

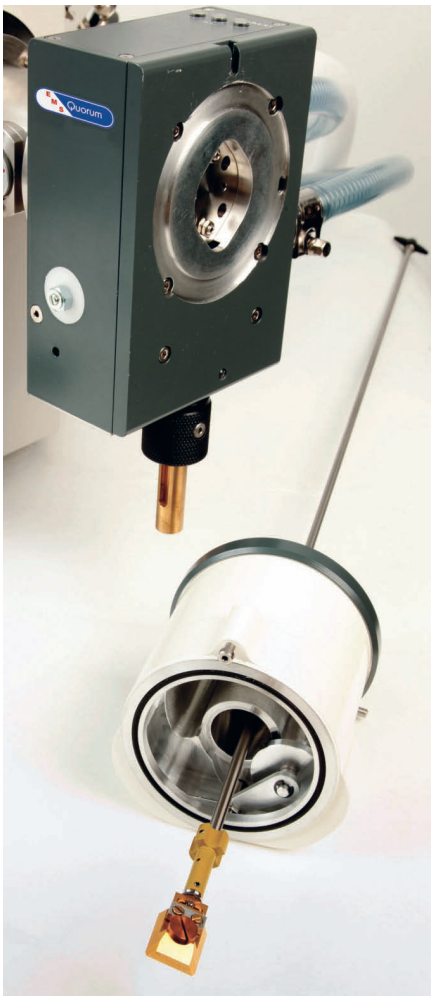
The QuickLok provides a rapid way of transferring ambient temperature specimens into SEM, FIB/SEM or other suitable vacuum systems. A key feature of the QuickLok is the ability to vacuum transfer specimens that are sensitive to normal environmental conditions. The transfer device uses a sealed vacuum chamber which can be interfaced to a glove box for inert gas transfer or allow vacuum transfer from a wide range of platforms.

#### Key Features

- Rapid specimen exchange
- Vacuum and inert gas transfer
- Field-retrofitable to most systems
- Upgrade path to CoolLok
- Custom designed holders available
- 3 year warranty



Specimen Holder Examples



QuickLok and specimen transfer device

#### Components

Mounted onto a suitable vacuum chamber port, the QuickLok consists of a loading chamber body with integrated controls for pumping, venting and transfer. A custom-designed interface flange and connections to the pumping system are included (see Pumping below).

The compact vacuum transfer device has an easy-release bayonet fitting to a dovetail-profile specimen holder (shuttle). Standard shuttles are included, but optional holders allow a range of specimen types to be handled.

Inside the microscope is a stage to accept the specimen shuttle. To aid specimen exchange an interlocked LED chamber light is mounted to the inside of the QuickLok interface.

#### Use

The specimen is mounted on a suitable holder and the transfer device fitted onto the QuickLok. The airlock and transfer device are then evacuated to a pre-set vacuum and the gate valve opened. The specimen is then guided onto the microscope stage.

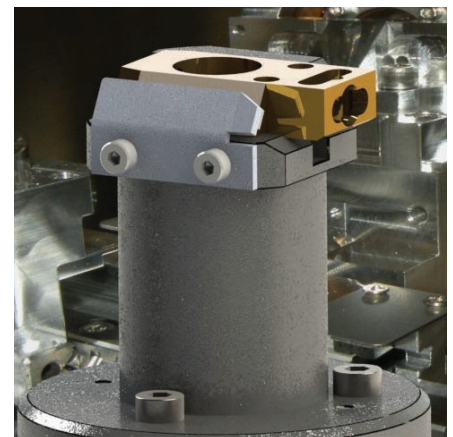
For transfer from other vacuum systems, or a glove box, additional interface flanges are available on request.

#### Pumping

The QuickLok requires either a rotary pump or oil-free vacuum turbomolecular pumping station (see Options).



Simple controls for specimen exchange



QuickLok specimen stage and adaptor to SEM



### III Cryo Transfer Systems (continued)

#### PP3005 SEMCool

*Non-airlock cryo cooling for SEM, FIB/SEM, beamline and vacuum platforms*

##### Quick Overview

The SEMCool is based on the PP3006 CoolLok but without the PP3004 QuickLok components. It is designed for cryogenic applications where airlock exchange of specimens into the microscope is not required.

##### Key Features

- Temperature range down to  $-190^{\circ}\text{C}$ , with stability better than  $0.5^{\circ}\text{C}$
- Off-column cooling with all-day runtime between fills
- Independent cooling of cold stage and cold trap
- Upgrade path to CoolLok
- 3 year warranty

##### Components

**Specimen holders and transfer device:** The compact vacuum transfer device has an easy-release bayonet fitting to a dovetail-profile specimen holder (shuttle). Standard shuttles are included, but optional holders allow a range of different specimen types to be handled.

**Cold stage and cold trap:** A highly stable, thermally isolated, nitrogen gas-cooled cold stage attaches to the microscope stage. The location and shape of the cold trap is tailored to suit the internal geometry of the microscope. Both cold stage and cold trap are capable of reaching temperatures down to  $-190^{\circ}\text{C}$  with a stability of  $<0.5^{\circ}\text{C}$ . For easy specimen exchange an LED chamber light is fitted.

The cold stage connects to the microscope stage using an adaptor and has a dovetail fitting to accept a specimen holder. When not in use the cold stage is uncoupled and stored within the chamber with the gas and electrical fittings connected.

**Cooling dewar, trolley and controller:** The cold stage and cold trap are cooled by a remotely-positioned, vacuum isolated 21 L dewar and heat exchanger assembly which at normal operating temperatures can run for up to 24 hours between fills. The gas lines between the dewar and the microscope interface are vacuum isolated for maximum thermal efficiency.

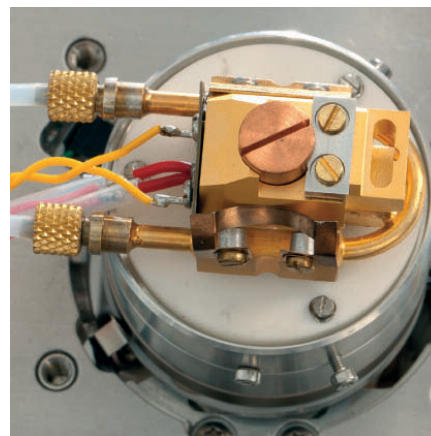
The cooling dewar sits on a floor-mounted trolley which also houses the monitor/controller for cold stage and monitor for cold trap, plus nitrogen gas flow controllers.

##### Use

Vent the SEM, locate specimen holder on the cold stage, re-pump the SEM and then cool down to the required temperature. To exchange specimen, warm to above  $0^{\circ}\text{C}$  and vent the SEM.

##### Pumping

The SEMCool requires a rotary pump to periodically evacuate the vacuum isolated lines (see Ordering Information).



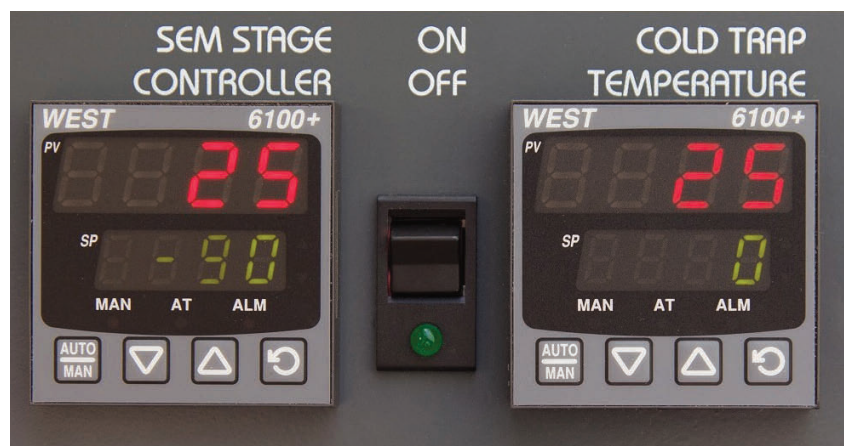
PP3005 SEMCool



Cold trap - adapted to installation

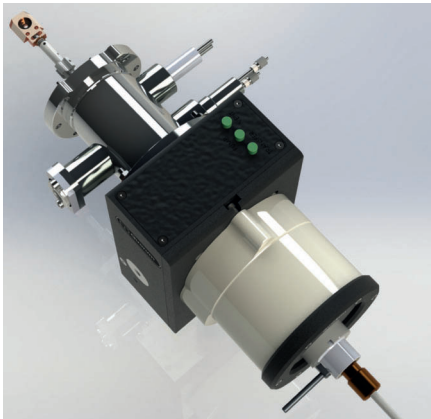


Controller and cooling system

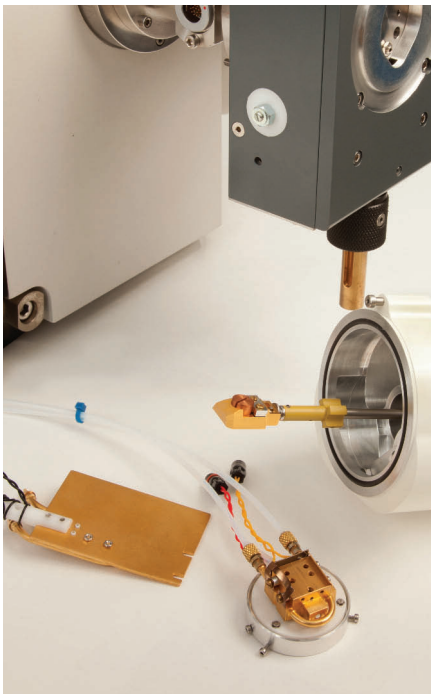


Temperature controller





PP3006 CoolLok



On-microscope components: airlock, cold stage, cold trap plus cryo transfer device



Load lock with vacuum isolated gas cooling lines

### III Cryo Transfer Systems (continued)

#### PP3006 CoolLok

*Cryo transfer systems for SEM, FIB/SEM, beamline and vacuum platforms*

##### Quick Overview

The CoolLok offers rapid transfer and cryo temperature observation of specimens for SEM, FIB/SEM, beamline or other vacuum systems. Applications include thermal protection of beam-sensitive specimens and low temperature observation of materials such as plastics, polymers low-K dielectrics and hard-soft mixtures. The system can also be used for inert gas transfer of ambient temperature specimens from a glove box.

Please Note: The PP3006 is not a replacement for the PP3010, which is a full cryo preparation system. The PP3006 does not have a cryo preparation chamber and is designed for materials applications where cold fracturing and sputtering are not required.

##### Key Features

- Rapid specimen exchange
- Temperature range down to  $-190^{\circ}\text{C}$  with stability better than  $0.5^{\circ}\text{C}$
- Off-column cooling with all-day runtime between fills
- Independent cooling of cold stage and cold trap
- Vacuum or inert gas transfer
- Rapid specimen freezing option
- 3 year warranty

##### Components

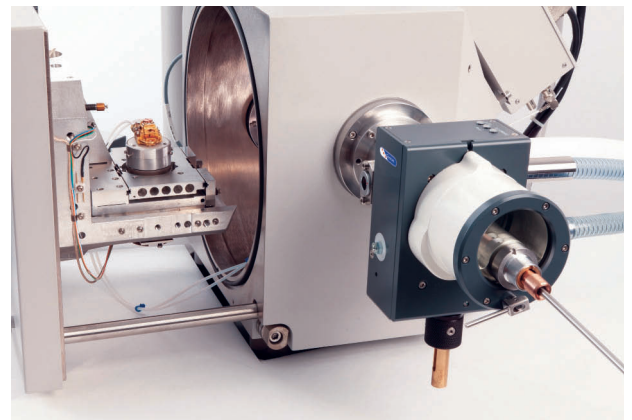
###### Vacuum airlock cold gas feedthrough

Mounted onto a suitable vacuum chamber port, the CoolLok consists of a loading chamber body with built-in controls for pumping, venting and transfer. A custom-designed interface flange to the vacuum chamber and connections and fittings to the pumping system are included (see Pumping below). The interface has cold nitrogen gas feeds to and from the microscope cold stage and cold trap.

###### Specimen holders and transfer device

The compact vacuum transfer device has an easy-release bayonet fitting to a dovetail-profile specimen holder (shuttle). Standard shuttles are included, but optional holders allow a range of different specimen types to be handled.

**Gold stage and cold trap** A highly stable, thermally isolated, nitrogen gas-cooled cold stage attaches to the microscope stage. The location and shape of the cold trap is tailored to suit the internal geometry of the microscope. Both cold stage and cold trap are capable of reaching temperatures down to -



PP3006 installation example



### III Cryo Transfer Systems (continued)

#### PP3006 CoolLok (continued)

190°C with a stability of  $<0.5^{\circ}\text{C}$ . For easy specimen exchange an LED chamber light is fitted.

The cold stage connects to the microscope stage using an adaptor and has a dovetail fitting to accept a specimen holder. When not in use the cold stage is uncoupled and stored within the chamber with the gas and electrical fittings connected.

**Cooling dewar, trolley and controller** The cold stage and cold trap are cooled by a remotely-positioned, vacuum isolated 21L dewar and heat exchanger assembly which at normal operating temperatures can run for up to 24 hours between fills. The gas lines between the dewar and the microscope interface are vacuum isolated for maximum thermal efficiency.

The cooling dewar sits on a floor-mounted trolley which also houses the monitor/controller for cold stage and monitor for cold trap, plus nitrogen gas flow controllers.

**Rapid freezing station (24429)** With the standard CoolLok, specimen freezing is by contact with the microscope cold stage following transfer and therefore freezing rates are relatively slowly. This is suitable for hard, non-hydrated specimens, but for liquid-based material rapid freezing is essential to reduce the detrimental effects of ice crystal growth and to allow through-vacuum transfer onto the cold stage.

For these applications the optional nitrogen slush freezing station is required. However, for many applications (especially lifesciences) cold fracturing and sputter coating are essential process steps and require the advanced capabilities of the EMS PP3010 – a full cryo preparation system.

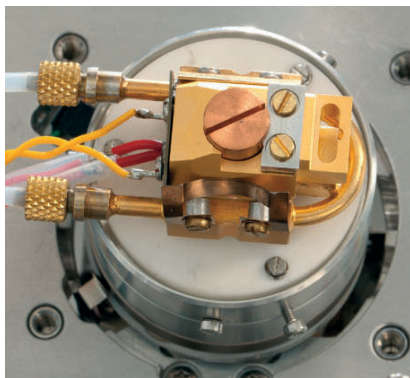
#### Use

The specimen is mounted on a suitable holder (shuttle) and the transfer device fitted onto the airlock and the dead space evacuated to a pre-set vacuum level. The gate valve is opened and the specimen guided onto the SEM stage.

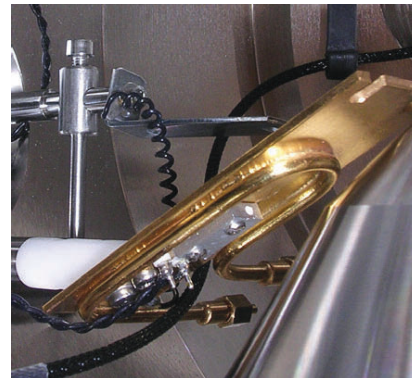
For transfer from other vacuum systems, or a glove box, additional interface flanges are available on request. Vacuum transfers can be made from the optional 24429 trolley-mounted nitrogen slush freezing station, if fitted.

#### Pumping

The QuickLok requires either a rotary pump or oil-free turbomolecular pumping station (see Options).



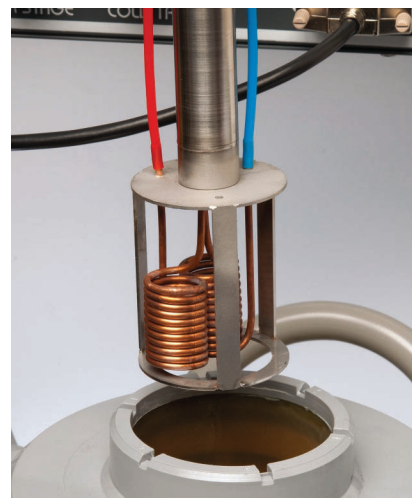
*Cold stage*



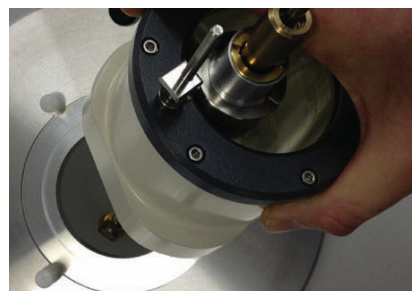
*Cold trap - adapted to installation*



*Dewar and Controller*



*Heat exchanger*



*Plunge freezing in slushy nitrogen*



### III Cryo Transfer Systems (continued)

#### SPECIFICATIONS for PP3004, PP3005, PP3006

	PP3004	PP3005	PP3006
<b>Temperature</b>	Ambient	RT to -190°C	RT to -190°C
<b>Cooling Runtime</b>	N/A	Up to 24 hours	Up to 24 hours
<b>LN<sub>2</sub> Dewar Capacity</b>	N/A	21 liters	21 liters
<b>Cool-Down Time to -190°C</b>	N/A	Typically <15 minutes	Typically <15 minutes
<b>Rapid Freezing (slushy LN<sub>2</sub>)</b>	N/A	Optional (24429)	Optional (24429)
<b>Dewar Trolley Footprint</b>	N/A	50 x 50 cm	50 x 50 cm
<b>Airlock Weight</b>	2.5 kg	2.5 kg	2.5 kg
<b>Pumping Requirements</b>	Rotary pump or dry pump	Rotary pump or dry pump	Rotary pump or dry pump
<b>Nitrogen Gas</b>	For venting and valve operation	Venting and cooling	Venting and cooling
<b>Power Requirements (excluding pump)</b>	300 W	300 W	300 W
<b>Maximum Specimen Size</b>	Flat specimens up to 23 x 26 mm. For taller specimens the maximum height will reduce from a mid-point of 9mm. Please contact us for more details.		

#### ORDERING INFORMATION *For a full quotation, including on-site installation and customer training, please contact us.*

**PP3004 QuickLok Ambient Temperature Transfer System**  
**Includes:**  
 Airlock assembly. Pump and vent and transfer controls, valve and fittings to the pumping system (see: Pumping below). Custom designed interface flange to the microscope vacuum chamber  
 Microscope dovetail stage to accept specimen shuttle.  
 LED chamber light (interlocked)  
 Specimen transfer device for vacuum or inert gas transfer  
 Specimen holders. Specimen shuttle with holding clips, specimen shuttle blank, specimen shuttle (to hold a 10mm dia. specimen stub), blank 10 mm stubs – packet of 10 each

**PP3005 SEMCool Non-Airlock Low Temperature System**  
**Includes:**  
 Nitrogen gas cooled cold stage with heater and sensor and cold trap with temperature sensor. Temperature controllable with a range down to -190°C, 21 L liquid nitrogen dewar with trolley, heat exchanger and LED chamber light. Pump fittings (see: Pumping below).  
 Temperature and nitrogen gas flow controller mounted on the dewar trolley.  
 Specimen holders. 3 specimen shuttles (to hold 10 mm Ø cryo stubs), blank specimen shuttle, specimen shuttle with holding clips, blank 10 mm Ø stubs (packet of 10), 5 multi-purpose specimen stubs. Note: other holders available  
 Specimen mounting compounds (colloidal graphite and Tissue-Tek®) each

**PP3006 CoolLok Cryo Transfer System**  
**Includes:**  
 Airlock assembly. Pump and vent and transfer controls, gate valve and fittings to the pumping system (see: Pumping below). Custom designed interface flange to the microscope vacuum chamber.  
 Cooling system. Nitrogen gas cooled cold stage with heater and sensor and cold trap with temperature sensor. Temperature controllable with a range down to -190°C, 21 L liquid nitrogen dewar with trolley, heat exchanger and LED chamber light.  
 Specimen transfer device  
 Specimen holders. 3 specimen shuttles (to hold 10 mm Ø cryo stubs), blank specimen shuttle, specimen shuttle with holding clips, blank 10 mm Ø stubs (packet of 10), 5 multi-purpose specimen stubs. Note: other holders available  
 Specimen mounting compounds (colloidal graphite and Tissue-Tek®), interlock cable and pump fittings each

#### Pumping

The PP3004 QuickLok and PP3006 CoolLok require either a rotary pump or high vacuum turbomolecular pumping station (recommended). The PP3005 requires a rotary pump for evacuating the vacuum isolated gas lines.

<b>13034</b>	Pfeiffer Duo 6 — 5 m <sup>3</sup> /hr rotary vacuum pump with oil mist filter	each
<b>24426</b>	Pfeiffer HiCube 80 turbomolecular and diaphragm pumping system	each

#### Options and Accessories

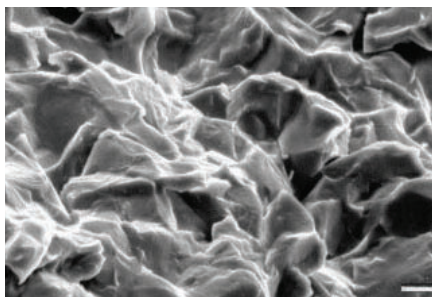
<b>24429</b>	Rapid cooling station (for PP3006 only) Consists of a floor-mounted trolley, liquid nitrogen freezing chamber mounted into the work surface which interfaces to the cryo transfer device, connections to vacuum pump (order separately)	each
<b>PP7450</b>	Pressurized (60 L) LN <sub>2</sub> dewar. Boil-off nitrogen gas is used for cooling the stage and cold trap (PP3005 and PP3006 only)	each
<b>13296</b>	Sircal in-line gas dryer. Helps to reduce water content of nitrogen gas supply	each

#### Specimen Holders

<b>10245</b>	Top-loading specimen shuttle for planchettes	each
<b>10246</b>	Top-loading specimen shuttle, to take a 10mm stub	each
<b>10247</b>	Top-loading specimen shuttle for rivets (vice style)	each
<b>E7433</b>	Rivet holder specimen stub, screw-down style (for use with 10246)	each
<b>E7449-5</b>	Universal specimen stub with surface holes and slots (5 pack)	each
<b>E7401</b>	Specimen stub shuttle (spare)	each
<b>E7402</b>	Aluminum (Al) stubs (10 pack)	each
<b>E7403</b>	Copper (Cu) stubs (10 pack)	each
<b>E7405</b>	Screw down stub for thin, hard specimens	each
<b>E7406</b>	Copper (Cu) stubs with 3 x 3mm slots (5 pack)	each
<b>E7407</b>	Copper (Cu) stubs with 1 x 3mm slot (5 pack)	each
<b>32816510</b>	Brass rivets for fracturing liquids (100 pack)	each

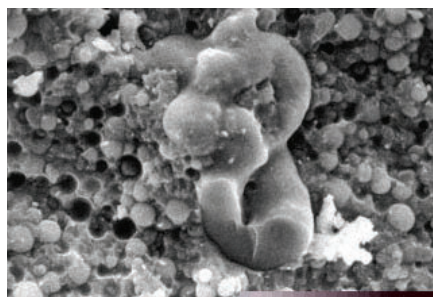
#### Sputter Targets and Carbon Fiber

<b>E7400-314A</b>	Gold (Au) target 0.008" thick	each
<b>E7400-314B</b>	Gold/palladium (Au/Pd) (80:20) target 0.2mm thick	each
<b>E7400-314C</b>	Platinum (Pt) target 0.008" thick	each
<b>E7400-314IR</b>	Iridium (Ir) target 0.008" thick	each
<b>E7400-314CR</b>	Chromium (Cr) target 0.3mm thick	each
<b>91047-1</b>	Carbon fiber cord — high purity — 1m	each
<b>91047-5</b>	Carbon fiber cord — high purity — 5m	each



**Wax crystals in gas oil**

When cooled to a temperature below about 2°C, the waxes in fuel oils such as this tend to crystallize out. Wax crystal size and shape can be varied by altering the rate at which the oil is cooled.



**Stable emulsion of a hydrophobic polymer**

This image illustrates a stable emulsion of a synthetic liquid polymer dispersed in an aqueous continuous phase.

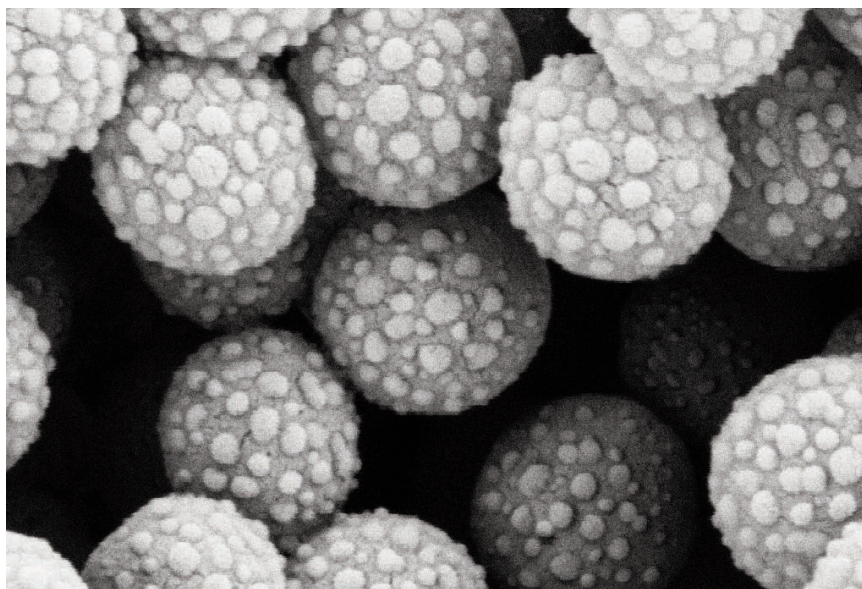


**Dendritic Ice Crystals**

If it is cooled slowly, water forms dendritic ice crystals. These can have a variety of branching patterns — the complexity of which depends upon cooling rate. Arms extend from the main body of the crystal at an angle of 60°. Some, such as the one illustrated, resemble the arms of a snowflake. Bar: 2µm



200 nm EHT = 5.00 kV Signal A = SE2 Date :7 Mar 2014  
 WD = 6.8 mm Mag = 50.00 K X File Name = Sample 062\_17.tif University of Brighton



100 nm EHT = 5.00 kV Signal A = SE2 Date :7 Mar 2014  
 WD = 6.8 mm Mag = 200.00 K X File Name = Sample 062\_15.tif University of Brighton

**Latex**

Latex particles are very electron beam sensitive, so cryo-SEM is an ideal method for their observation



# Cryo-SEM Applications

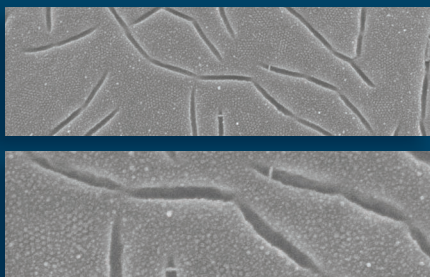
## Zoological



### Frozen hydrated aphid

In comparison with the critical point dried aphid, this image shows that there is no distortion of the abdomen nor any other parts of the aphid following freeze drying.

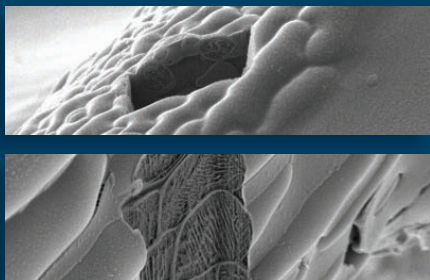
## Fungi



### Baker's yeast (*Saccharomyces cerevisiae*)

The specimen was rapidly frozen in nitrogen slush, fractured and coated with 4nm of platinum (Pt). 10nm yeast cell transmembrane particles (in hexagonal arrays) can be observed.

## Cryo-DualBeam



### Arabidopsis plant

Cryo-FIB/SEM. Image courtesy of Hannah Edwards and Arabidopsis plants provided by Darren Wells, Centre for Plant Integrative Biology, School of Biosciences, University of Nottingham, UK.

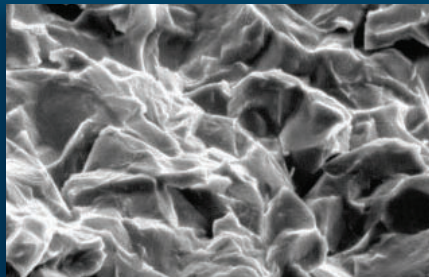
## Botanical



### Pollen of cactus *Zygodactylus truncatus*

Germinating pollen grains of *Zygodactylus truncatus*.

## Geological



### Wax crystals in gas oil

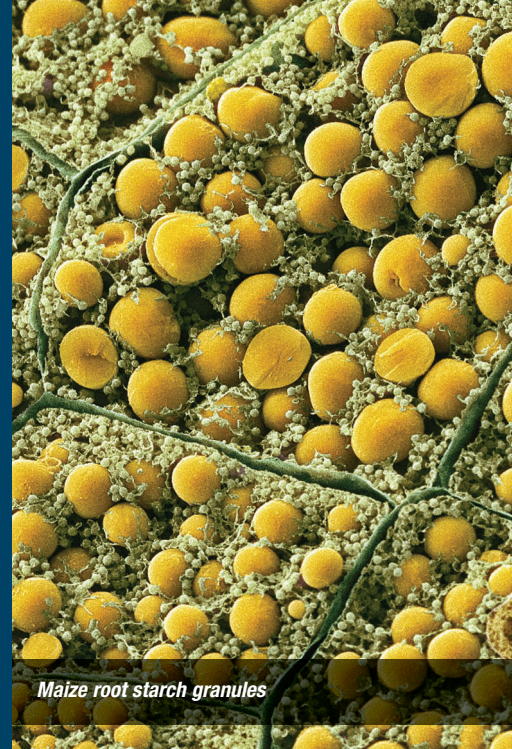
When cooled to a temperature below about 2°C, the waxes in fuel oils such as this tend to crystallize out. Wax crystal size and shape can be varied by altering the rate at which the oil is cooled.

## Polymers



### Stable emulsion of a hydrophobic polymer

This image illustrates a stable emulsion of a synthetic liquid polymer dispersed in an aqueous continuous phase.



Maize root starch granules



Predatory Mite, courtesy of USDA Beltsville, Dr Gary Buchan

## Electron Microscopy Sciences

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