User Guide to Alto Cryo-SEM
Cryo Transfer Scanning Electron Microscope (Cryo-SEM)

Cryo-SEM is now a widely used technique for the observation of many sample types from a host of different academic, R&D and quality control facilities for example, botanical, biological, agricultural, pharmaceutical, petrochemical, food, cosmetic, detergent and construction industries. Cryo-SEM has two main advantages over other sample preparatory techniques; samples remain hydrated and preparation time is extremely short, typically 15 minutes or less. A modern FE-SEM equipped with Alto 2500 has enabled Cryo-SEM to be regarded as a high resolution technique, with the visualization of structures less than 5nm to be achievable at low accelerating voltage.

**Use of cryo-SEM:**

Many materials are sensitive to the vacuum conditions and/or the high electron beam energy in the SEM. These include biological and other ‘hydrated’ materials, also low melting point or volatile specimens, even liquids. In addition materials which are normally soft at room temperature can be fractured under cryogenic conditions to expose internal microstructure and the dispersion of components and phases in a system such as an emulsion or suspension. A “process” or (setting) can be observed as a time resolved series of frozen samples.

**Applications of cryo-SEM:**

All of the biological sciences, especially botany, mycology, agricultural sciences, biotechnology and biomedical. Related to the above are applications in the pharmaceutical, healthcare and cosmetics industries, also R&D and QA of products e.g. drug delivery, cream preparations, dressings. Applications in food technology including emulsions, suspensions, multi-phase products e.g. ice cream, dairy products, texture, keeping properties and spoiling organisms. Other industries using cryo-SEM include oil, chemical, paper and other forest products, textiles, paint, printing and cement.

**Advantages of cryo-SEM over other preparation techniques:**
Chemical fixation is avoided; a cryo-SEM sample, rapidly frozen, is as close as possible to its natural state. No use of solvents, which can also remove sample components. No dehydration, delicate structures are maintained without shrinkage. Fast freezing means chemical balance is well maintained for microanalysis. A soft, volatile or liquid sample is stabilised under the electron beam. Freeze fracture and controlled freeze etching allow optimum exposure of internal structure. Cryo-SEM is fast – typical preparation time is less than 10 minutes!

**Cryo-SEM technique:**

The sample is mounted on to the appropriate sample holder and then plunge frozen, usually into ‘slushy’ nitrogen. The sample holder is then withdrawn, under vacuum, into a vacuum transfer device for transfer to the cryo-preparation chamber. After transfer to the (separately pumped) cryo-prep chamber the sample is maintained at a low temperature and low contamination conditions. The sample may be fractured and/or freeze etched (by controlled raising of the temperature until sublimation can occur) to expose internal structure. Finally a thin conductive coating is usually applied to allow high resolution imaging or microanalysis in the SEM. Transfer to the SEM chamber is via an interlocked airlock and onto a cold stage module fitted to the SEM stage.

**We have a VP ‘Natural’ SEM or FESEM. Why do I need cryo?**

Cryo-SEM is still the best way to prevent dehydration, which will occur at any vacuum levels and is difficult to control even with Peltier stages and water vapour in the SEM chamber. Cryo- allows freeze-fracture and the exposure of internal structure. It also stabilises soft materials and liquids which would otherwise be impossible to examine at high magnification, due to sample movement or beam damage. Despite the versatility of the above EMs in imaging un-coated specimens, there are often advantages in applying a thin, high quality sputter coating – this allows better surface imaging (especially of biological materials) and higher resolution results. **Alto 2500** is a perfect complement to these modern, flexible SEMs.

**Wel have a FE SEM. Can cryo give me high resolution results?**

Yes! **Alto 2500** was designed specifically to provide maximum resolution results on all models of FEG SEM. There are many factors to achieving this, including: Contamination-free sample preparation, within a cold-shielded, high vacuum cryo-preparation chamber. Leading technology cold magnetron sputter coater, with a range of target materials. High stability, gas-cooled, cold stage module. Anti-vibration designed into the whole system, FEG SEM performance is not compromised even with the cryo-system operational, pumping system running. All this results in a typical resolution of 5nm achievable on a cryosample using **Alto 2500**.

**What about microanalysis using cryo-SEM?**

As previously mentioned, stabilisation of the sample under the beam conditions needed for x-ray microanalysis makes cryo-SEM an ideal technique. Both **Alto 2500** and **Alto 2100** can provide the popular Cr sputter coating technique to prepare the cryo-specimen for microanalysis.

**How is ALTO the “next generation” cryo-system?**

Gatan UK, previously the EM Products Group of Oxford Instruments, has more than 15 years experience of
manufacturing state-of-the-art cryogenic systems for SEM. *Alto* not only comes from that technical expertise but was also designed to radically improve the ‘ease of use’ of cryo-SEM. This is typified by the replacement of the typical bulky and complex boxes of control electronics with a small neat deskpad controller which can be moved by the user to wherever is convenient. *Alto* is also ‘future proofed’ in that the cryo-preparation chamber has been designed to allow easy upgrades to whatever new techniques become available.

**The Procedure step by step:**
Cryo-SEM has three principal operational phases and the basics of the technique are simple.
Phase one: The sample is mounted onto a suitable sample mount attached to a sample holder. Large selection of sample holders to choose from.

Step 1 This sample is attached to a stub.

Step 2 The stub is inserted into holder.

Step 3 The holder is then attached to the transfer rod.

Step 4 The sample holder is then cooled quickly by plunging into liquid nitrogen slush.
Step 5 The sample is then transferred under vacuum to the preparation chamber. Sample now held under vacuum in transfer device following plunge cooling ready for phase two.

Phase two:
Step 6 The transfer device is attached to the preparation chamber and the sample holder transferred to the cold stage.

Step 7 In the preparation chamber the sample may be fractured, to expose internal microstructure.

Phase three: Step 8 The sample is transferred to the SEM chamber and observed at low temperature.

C r y o P r e p a r a t i o n  f o r  t h e  S E M :  

Cryo preparation has become the preferred technique for preparing any hydrated or low melting point material for the SEM. It is non-destructive and allows imaging and analysis of biological and beamsensitive or vacuum-sensitive specimens. It offers several advantages over more conventional techniques – it can be completed within a few minutes, chemical fixation and contact with solvents is avoided, levels of specimen hydration are maintained, low melting-point materials are stabilised, volume changes are minimised and internal structure can be revealed by freeze-fracture.

In addition, elements are not lost or substantially relocated prior to elemental X-ray microanalysis. As well as studies of biological material and microorganisms, the technique allows ‘unstable’ multiphase systems, emulsions and suspensions to be examined rapidly. This also allows dynamic processes to be characterised in frozen ‘snapshots’. Internal microstructure to be exposed by cold-fracture, providing insight and information not accessible by simply imaging the surface of a sample.

Life Science applications include botanical and physiological research, biotechnology and agrochemicals. Cryo-SEM is also important for R&D, quality assurance and problem-solving in the pharmaceutical, healthcare, food, chemical, oil and cement industries.
With cryo preparation, specimens are usually rapidly frozen, initially in cryogenic ‘slush’ and transferred under vacuum through an air lock to a cryopreparation chamber attached to the SEM. Freeze fracture, etching and fine metallic coating (providing sample conductivity to enable the highest quality imaging) are performed in the cryo-preparation chamber, which also includes active sample cooling.

After preparation, samples are transferred to the main SEM specimen chamber and mounted on a high stability nitrogen gas cooled cold stage, ready for SEM examination and microanalysis. The entire process, from initial freezing to readiness for SEM highresolution imaging, typically takes less than 10 minutes.

A Modern Cryo-SEM System:

Gatan’s ALTO range of cryotransfer systems is a new generation which enhances the capabilities of all models and types of SEM including Field Emission, Low Vacuum, Variable Pressure, Environmental and High Vacuum. Specimen preparation techniques provided with every ALTO system include rapid freezing (to preserve hydrated specimens), vacuum transfer (to prevent contamination), cold fracture (to expose internal microstructure), freeze etching (to clarify components) and coating (to allow high resolution surface imaging and X-ray microanalysis). The ALTO range enables optimum imaging and analysis, even of difficult specimens, in the SEM and is chosen by industrial and research organisations for all SEM platforms. ALTO systems use a high-stability SEM cold stage and state-of-the-art cold magnetron coating technology, together with a high vacuum, low contamination cryo-preparation chamber. This combination allows very high imaging quality, typically to 5nm resolution in a Field Emission SEM.

Preparation technology also includes variable temperature control (to sublime or ‘etch’ away ice, exposing further microstructure) and a choice of cold fracturing devices. ‘Visibility’ and ‘ease of use’ are important themes, so the ALTO features a completely new system control ‘keypad’. This can be placed wherever is convenient for the operator, leaving power electronics out of the way.

Users wishing to remain at the forefront of Cryo-SEM technology in years to come can be assured that the ALTO design will accommodate future technologies and upgrades, which can easily be added as needed.