



## Frequently Asked Questions (FAQ) *Cryo-SEM and Alto systems*

### ***Why use 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.

### ***What are some of the applications of cryo-SEM ?***

All of the biological sciences, especially botany, mycology, agricultural sciences, biotechnology and bio-medical.

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.

### ***What are the 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 !

***Briefly, what is the 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The sample holder is then withdrawn, under vacuum, into a vacuum transfer device for transfer to the cryo-preparation chamber.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.

***I have a LV / CP / VP / 'Natural' SEM or ESEM. 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 2100** is a perfect complement to these modern, flexible SEMs

***I have a FEG 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 cryo-sample 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.

***I want to examine pre-frozen specimens. Has this been considered ?***

Yes, both **Alto** systems come with a 2 pot freezing station plus a special device to assist the loading of pre-frozen samples onto the **Alto** sample holder, before vacuum transfer.

***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.