OBSERVATIONS OF SNOW AND ICE CRYSTALS WITH LOW TEMPERATURE SCANNING ELECTRON MICROSCOPY (REVIEW)

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ABSTRACT

This review summarizes the advantages of LTSEM for observations of samples of snow and ice by illustrating the type of surface information that is obtainable, the resolution that can be attained and how the depth of field allows one to observe crystals with significant topography. In addition, we illustrate samples collected from remote locations, samples from time sequences during a storm and finally show examples of snow and ice biota as well as, artificial and Martian snow.

INTRODUCTION

The scanning electron microscope (SEM) has been widely used by biologists for nearly fifty years to study living organisms. The high resolution, which is less than 10 nm in modern instruments, and its depth of focus, which exceeds that of the common optical microscope by nearly 1,000 fold, provides detailed information and surface topography that is not obtainable with a light microscope.

In spite of this long history and these unique advantages, the SEM was first modified to image samples of snow and ice about ten years ago (Wergin and Erbe, 1994a; 1994b; 1994c). The conventional SEM stage, which operates at ambient temperature, was replaced with a stage that could be maintained at near liquid nitrogen temperatures. As a result, high resolution images of the surfaces of frozen specimens could be observed and photographed with a technique known as low temperature scanning electron microscopy (LTSEM).

During the past 10 years, the authors used this technique to re-examine the precipitation particles described in The International Classification for Seasonal Snow on the Ground (Colbeck et al., 1990). Their studies include observations of columns, needles, plates, stellar dendrites, irregular crystals, graupel, hail and ice pellets (Foster et al., 1997; Rango et al., 1996a; 1996c; 1997; 2003; Wergin et al., 1995a; 1995b; 1996a; 1996c; 1996d; 1998a; 1998b; 1999; 2001; 2002a; 2002b; 2003) as well as, metamorphosed snow (Foster et al, 1996; Rango et al., 1997; Wergin et al., 1996a; 1996b; 1996d; 2001; 2003), glacial ice (Erbe et al., 2003; Rango et al., 2000; Wergin et al., 1998a; 1998b), snow and ice biota (Rango et al., 2000; Wergin et al., 2005), and artificial (Wergin et al., 2005) and "Martian" snow (Foster et al., 1997; 1998; Wergin et al., 1997a). These studies are briefly summarized below.

MATERIALS AND METHODS

Sampling techniques

Details of the sampling procedures for snow crystals and ice grains were recently published by Erbe et al. (2003) and Wergin et al; (1997b). Briefly, all samples of snow and ice are collected on sampling plates, which are fabricated in the laboratory. The plates, which are cut from sheets of stock copper, 1.5 mm thick, measure 15 mm x 29 mm. At the sampling site, a plate is coated with a thin layer of a cryo-adhesive, such as Tissue Tek. The adhesive and the plates are cooled to temperatures at or slightly below freezing. Immediately after a specimen is collected, the plate is plunged into a vessel of liquid nitrogen (LN_2). In all subsequent procedures, including shipping, storing, coating, observing and photographing, the samples are maintained at near LN_2 temperatures (-196^{0}C). At these temperatures, the vapor pressure of water is not significant and sublimation does not occur at a detectable rate. Furthermore, re-crystallization does not occur (Beckett and Read, 1986) and fully frozen and hydrated samples remain stable for several hours while being observed in the LTSEM (Wergin and Erbe, 1991).

Paper presented at the Western Snow Conference 2006

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Falling snow is sampled either by allowing it to settle on the surface of the plate containing the cryo-adhesive or by lightly brushing a sample onto the plate. To collect samples from snow pits, a pre-cooled (LN_2) scalpel is used to gently dislodge snow crystals from a freshly excavated pit wall. The crystals are allowed to accumulate onto a plate and then plunged rapidly into the LN_2. To collect sintered or tightly bonded snow crystals from their native site, a LN_2 cooled scalpel blade is used to dislodge grain clusters from the pit wall. The clusters are collected onto a plate containing the cryo-adhesive and then frozen. This technique can also be used to sample wind slab, sun crust or depth hoar, which may exist in the stratified layers of the snow pit. The sample plates can also be placed in areas where rime, surface hoar or artificial snow is expected to occur. When a sufficient sample has sublimed or accumulated on the plate, it is plunge frozen in LN_2.

Fracturing snow clusters or glacial ice can be used to reveal the extent of internal air spaces, details of sintering or the presence of biota, such as ice worms, algae, fungi and bacteria. Fracturing is accomplished with a pick that is used to randomly remove a portion of the snow cluster or ice sample. This process exposes a pristine internal surface that is then coated and imaged in the LTSEM.

Crystals of carbon dioxide were produced in the laboratory by introducing CO_2 gas into the cryo-system and allowing the gas to sublime onto a plate that was cooled to near LN_2 temperature. This procedure could also be used to produce crystals of other gases, as long as they are stable at LN_2 temperature.

Shipping and Storing Samples

At the collection site, a forceps is used to insert plates diagonally into square brass tubing, 13 x 13 mm inside diameter. The tubes containing the sampling plates are lowered into lightweight dry shipping Dewar or Cryopak Shipper (Taylor Wharton, Theodore, AL 36582) that had been previously cooled with LN_2. The Dewar is carried from the collection site and then either transported by vehicle or sent by priority air express to our laboratory in Beltsville, MD. The shipper, which is designed to maintain LN_2 temperatures for a minimum of 21 days when properly pre-cooled, has been used to transport samples from numerous locations including remote regions of Washington, North Dakota and Alaska. Upon reaching the laboratory, the samples are transferred to a LN_2 storage Dewar where they remain until being further prepared for observation with low temperature SEM.

Coating Samples

All frozen samples are coated with 2 to 10 nm of platinum by using a magnetron sputter coating device in a high purity argon environment within the pre-chamber of the cryo-system.

Recording Images in the SEM

The commercial specimen holder, which is supplied with the cryo-system, was modified to accommodate the sampling plates. For observation, the specimen holder containing the plate is inserted into a Hitachi S-4100 field emission SEM (Hitachi High-Technology Corp., Tokyo, Japan) equipped with an Oxford CT 1500 HF Cryo-system (Oxford Instruments, Enysham, England). The cold stage is maintained at -130^0 to 185^0 C. Accelerating voltages of 500V to 10 kV are used to observe the samples. The samples are imaged for as long as two hours without observing any changes in the structural features or in the coating integrity of the snow crystals or ice grains. Selected images are recorded onto Polaroid Type 55 P/N film (Polaroid, Cambridge, MA, USA).

Stereo pairs can be obtained by recording one image, tilting the sample 6 degrees, re-centering the subject and then recording the second image. The two images resulting from this procedure contain the parallax information necessary for three-dimensional observation and study.

RESULTS AND DISCUSSION

Low Temperature Scanning Electron Microscopy (LTSEM)

From the time of collection through observing and photographing, the samples are either stored in liquid nitrogen (LN_2) or are maintained at near LN_2 temperatures (-180^0 C to -196^0 C) on cold stages. At these temperatures and storage times, sublimation would be less than 1.49×10^{-5} nm/sec (Umrath, 1983), a rate that would not be detectable or significant under our working conditions. As a result, no indication of sublimation or evaporation is associated with the collection, preparation and observation of samples in the LTSEM. Fresh snow that was collected from remote sites, shipped and stored in the laboratory before observation exhibited the same structural features as fresh snow that was collected at the laboratory site and immediately observed in the low temperature SEM.

Surface Information

All samples observed with an SEM are coated with less than 10 nm of a heavy metal to prevent charging during examination and photographic recording with the instrument. The interaction between the primary electron beam generated in the SEM and the coating on the surface of the sample causes excitation of secondary electrons. These secondary electrons are collected and displayed on a screen (cathode ray tube) to form the image. As a result, this image only consists of upper surface information (Fig. 1). In the light microscope the images of snow and ice crystals generally consist not only the upper surface resulting from the reflected light, but the final images also contain information from reflected and refracted light from the undersides or the internal structure of crystals. Consequently, the true structure of the crystal surface can be difficult to perceive.

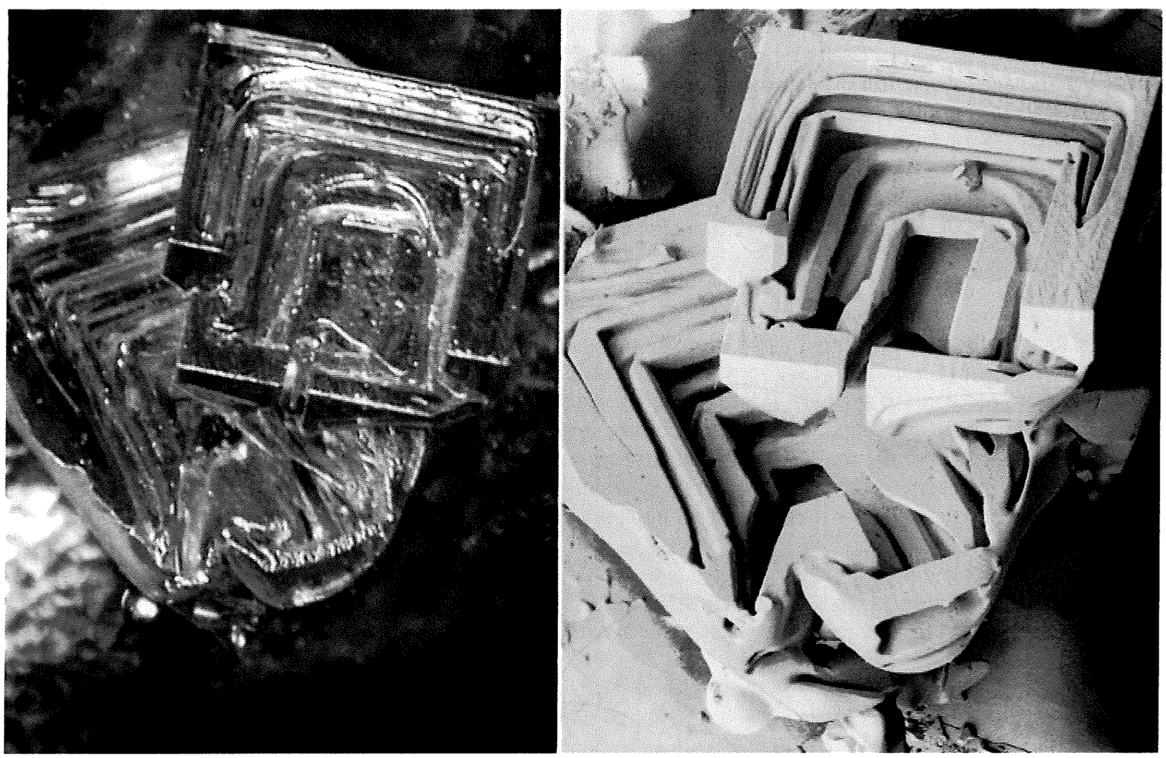

Figure 1. *LM and LTSEM images of an identical depth hoar crystal. The transmitted, reflected and refracted light that occurs in the LM images compromises the internal and external features of the crystal. The external features of the crystal are clearly evident in the LTSEM image. Magnification = 20X.*

Resolution

As a result of the technical difficulties of working with snow, most of the published photomicrographs that have been successfully taken with a light microscope, or photomacrographs taken with a camera, are published at magnifications of less than 400x (see Bentley and Humphreys, 1931; Magono and Lee, 1966; Nakaya, 1954). Alternatively, modern SEMs have useful magnification to at least 100,000X and resolutions of less than 5 nm. This feature has helped to illustrate and define surface detail (Fig. 2), the irregular crystals (Fig. 3), frequently alluded to by other investigators and to more clearly depict riming and graupel (Figs. 4 and 5). The full potential value of resolution for increasing our understanding of snow and ice has yet to be explored.

Depth of Field

Crystals with deep topography, such as aggregates, columns, graupel, and depth hoar are difficult to photograph with a light microscope because of a limited depth of focus. The depth of focus (Dfo) for the LM is dependent upon the magnification (M), resolving power (R) of the instrument and aperture angle (a) of the objective lens (Dfo = M2 x R/a). Simple calculations indicate that the LM has a depth of focus approximately 1/1000 that of a modern SEM. As a result, well focused images of large crystals with significant topography can be observed and photographed (Fig. 6).

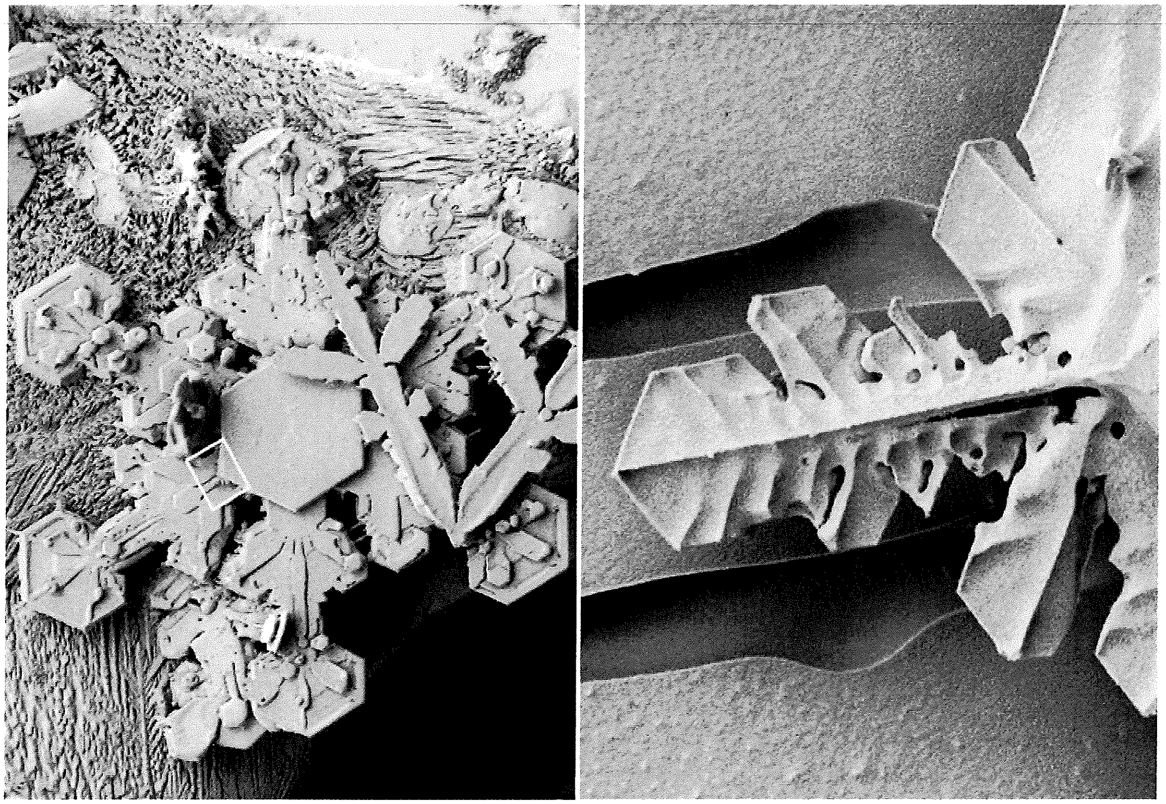

Figure 2. *Hexagonal dendrite collected at Bearden Mountain, West Virginia. A higher magnification image (right image 600X) is from the white rectangle shown in the left image. 30X.*

Remote Locations

Details of snow crystals metamorphosis and glacial ice are difficult to observe and document because these changes are frequently studied at remote locations and at altitudes that can be difficult to access with the appropriate instrumentation. Consequently, cameras (photomacrography) have been more widely used to document these snow grains than light microscopes (photomicrography) (La Chapelle, 1969). The procedures developed in our laboratory for collecting and transporting snow samples from remote sites have allowed us to characterize metamorphosed snow crystals and glacial ice from samples that have been collected at altitudes exceeding 10,000 feet and from distances of more than 2,000 miles from our laboratory (Figs. 7 and 8).

Time Sequences

Similarly, our collection procedures, which allow sampling and storing of hundreds of specimens from any one site, have allowed us to make systematic collections of snow during a storm event in an effort to illustrate the crystal types that occur over time (Figs. 9 and 10).

Snow Biota

In addition to snow and ice crystals, our procedures enable us to also study the natural biotas that are found in extreme environments. For example, the spring snowpack frequently develops a reddish hue and is commonly referred to as "red snow". The reddish hue results from the pigments present in a motile alga (*Chlamydomonas nivalis*) that can be found in the water film, which is present throughout the snowpack. Fracturing snow clusters that exhibit this coloration reveals the algae that populate the water film. The algae, which are unicellular and circular in fractured samples, measure approximately 20 μm in diameter (Fig. 11).

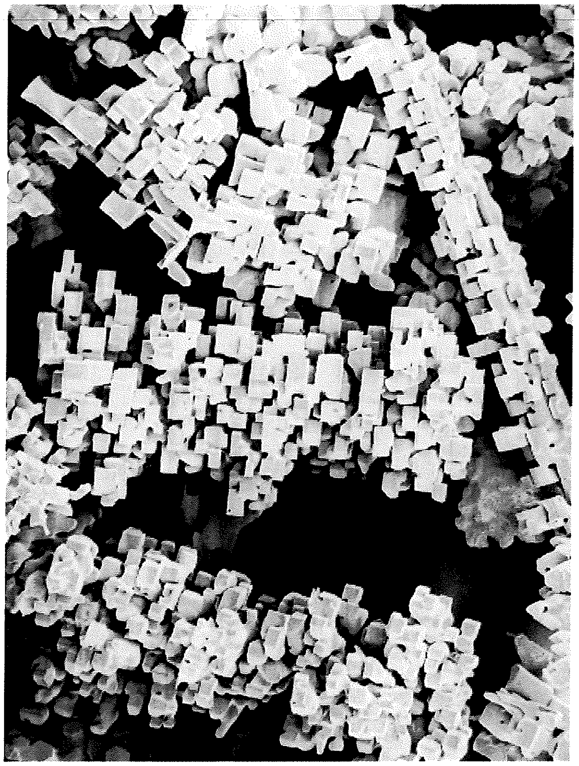

Figure 3. *Aggregate consisting of hundreds of irregular crystals. The crystals are hexagonal and have diameters that rarely exceed their height. 50X.*

Figure 4. *Hexagonal crystal in which one surface has become heavily rimed with frozen particles (cloud droplets). 50X.*

Imaging the surface layer of ice from glaciers, which represent early firn formation, reveals large, irregularly shaped ice grains, about 1 mm in diameter, that are sintered with adjacent grains. The continuous air spaces between the ice grains are prevalent at this stage. Unique organisms, commonly known as ice worms, can be found in the air spaces that exist in the firn (Fig. 12).

Artificial/Martian Snow

Natural snow generally forms when a nucleating particle triggers vapor deposition; a solid forms directly from the vapor or gas molecules. As we have seen, this process results in snow particles having distinct crystalline features. Alternatively, artificial snow, which is widely used for recreational purposes, forms when a liquid, that is near its freezing point and containing ice nucleating particles, is atomized under pressure into a cold atmosphere where the minute droplets quickly freeze. As a result, artificial snow grains appear as spherical grains of ice, similar to sleet, and do not possess any of the crystalline attributes of natural snow. The size of the droplets, which can vary from 0.1 to 1.0 mm, is largely a function of the pressure and type of gun that is used to atomize the water (Fig. 13).

Crystals of gases other than water vapor can also be imaged with LTSEM. Carbon dioxide gas, which was condensed on a pre-cooled sample plate, resulted in a solid condensate consisting of octagonal crystals that measure 10 to 15 µm (Fig. 14). These crystals of CO_2 frost are believed to be similar to those that comprise the seasonal polar caps of Mars.

Figure 5. *Graupel particle consisting of an aggregation of frozen cloud droplets. Original snow crystal cannot be discerned. 40X.*

Figure 6. *Blade of grass that is heavily encumbered with surface hoar or frost consisting of needle-like crystals. 25X.*

CONCLUSIONS

Snow may cover up to 53% of the land surface in the Northern Hemisphere (Foster and Rango, 1982) and up to 44% of the land areas of the world at any one time. This snow cover supplies nearly one third of the water that is used for the irrigation and the growth of crops (Gray and Male, 1981). For this reason, estimates of the quantity of water that is present in the winter snowpack are important to agriculture because they are related to the amount of moisture that will be available for the pending growing season. To achieve snow water equivalent estimates, scientists have used microwave remote sensing techniques on snowpacks prior to melting (Goodison et al., 1990; Rango et al., 1989; 1996a). Unfortunately, the estimates appear to be confounded by the shapes and sizes of the crystals in the snowpack. Therefore, increasing our knowledge of these factors may help to increase the accuracy of the models that are used for estimating snow water equivalents.

During formation and descent, snow can capture large quantities of particulate matter from the atmosphere (Iliescu et al., 2002). This occurs when atmospheric particles are scavenged by snow, a process known as "washout", or when atmospheric particles become attached or incorporated into the developing snow crystal, known as "snowout". Therefore, analysis of snow can be used to determine the nature and concentration of particulate matter that is present in the atmosphere. Gray and Male (1981) also suggest that because a snowflake falls at a slower rate and sweeps a larger area than a raindrop, snow has a greater exposure to pollutants and would be a better indicator of their presence. Perhaps combining LTSEM with an x-ray microanalysis would allow investigators to chemically characterize foreign particles in snow similar to the procedures that are currently being used in ice (Barnes et al., 2001; Cullen and Baker, 2000; 2002; Cullen et al. 2002; Dominé et al., 2001; Iliescu et al., 2002; Mulvaney et al., 1988; Wolff et al. 1988). This approach, which provides qualitative and semi-quantitative elemental composition of the particles, would provide a new tool for studying acid snow and air pollution.

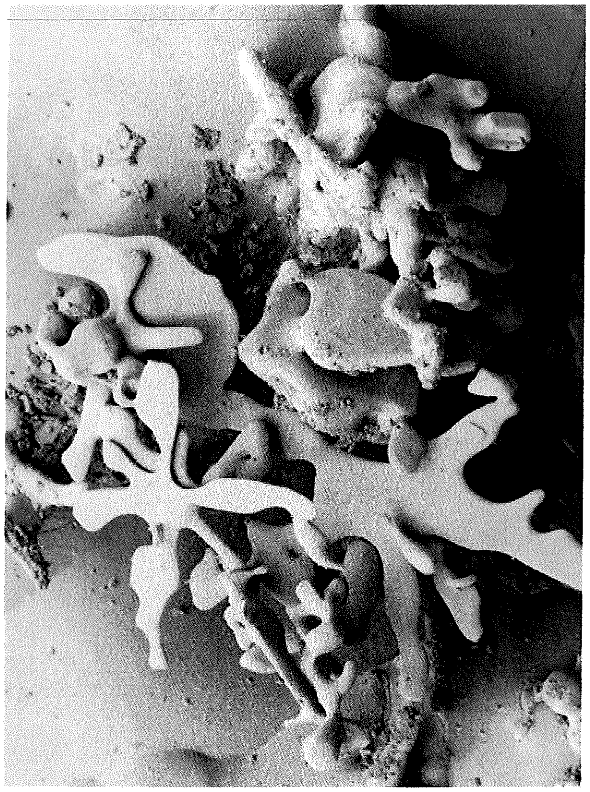

Figure 7. *Early stages of metamorphosed snow crystals that occur when snowpack is subjected to a small temperature gradient. The edges of the crystals loose their sharp crystalline characteristics and become rounded and bonded to neighboring crystals. 90X.*

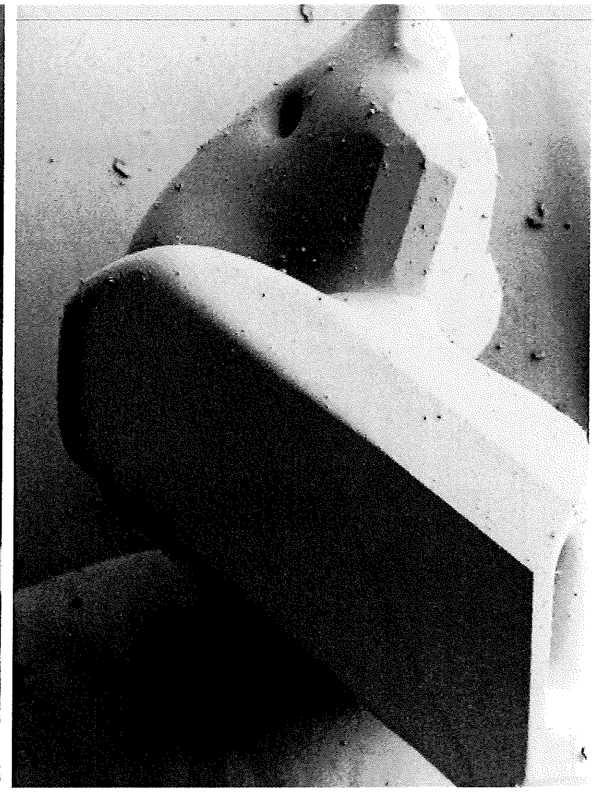

Figure 8. *Large crystal of depth hoar formed at the base of a snowpack that was subjected to a large temperature gradient. Sample collected in Wyoming. 100X.*

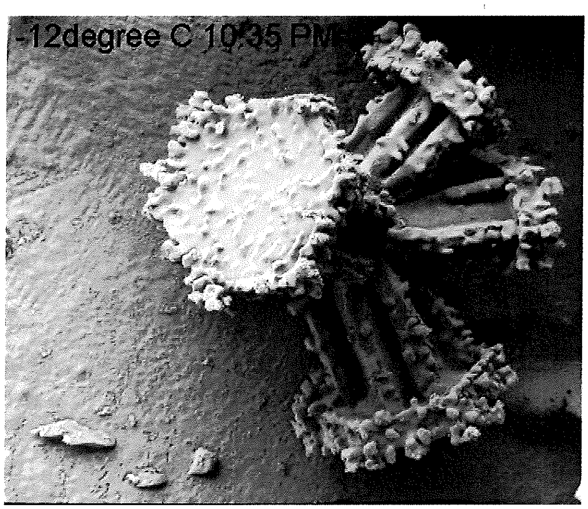

Figure 9. *Tetrad of bullets capped with hexagonal plates. The crystals, which are rimed, were collected during the early stages of a storm when the air temperature was -12^0 C. 65X.*

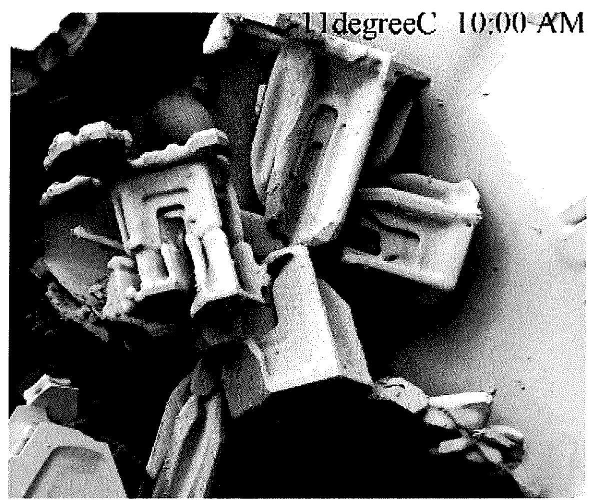

Figure 10. *Capped column and bullets that were collected from the same storm about 12 hours later than those shown in Figure 9. These crystals do not exhibit the riming shown in the earlier collection. 60X.*

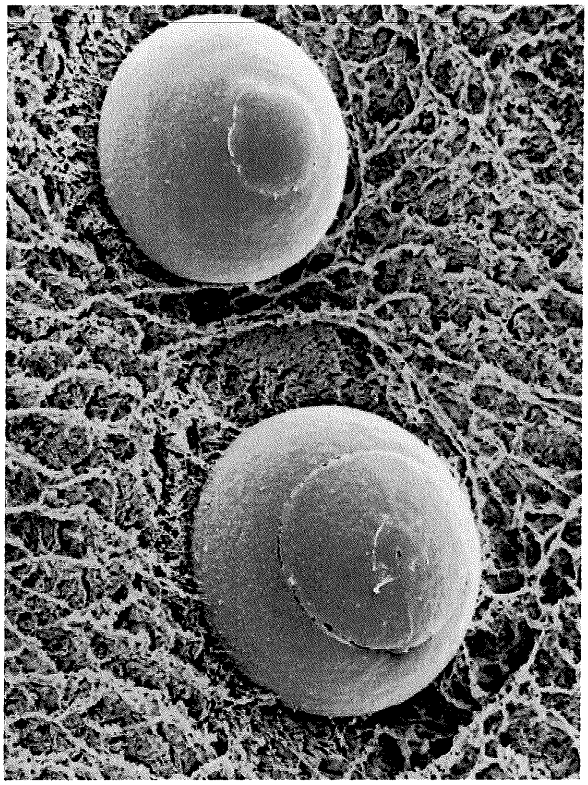

Figure 11. *Fracture of metamorphosed snow under freeze/melt conditions. The fracture reveals spherical bodies that represent flagellated algae frequently found in the film of free water in spring. 2000X.*

Figure 12. *Portion of an ice worm that frequents the air spaces illustrated in the early stages of glacial ice depicted. Sample collected at South Cascade Glacier, Washington. 15X.*

Figure 13. *Artificial snow collected in the plume of a snow gun. Artificial snow appears as small ice pellets. Sample collected at Sugarbush, Vermont. 50X.*

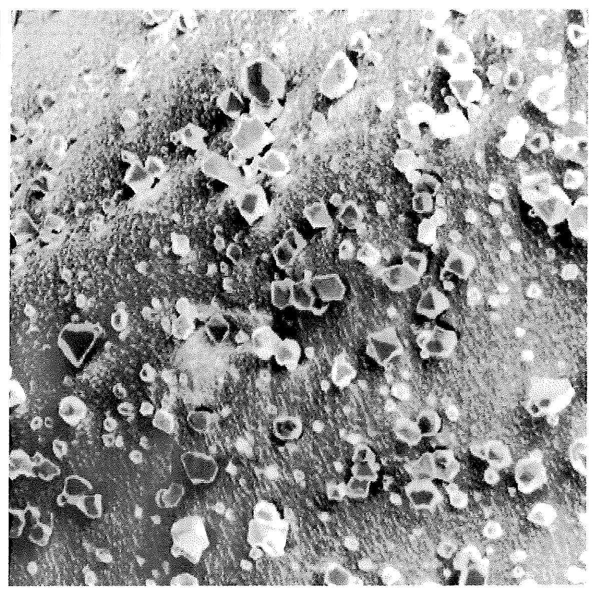

Figure 14. *Crystals of CO_2, that may simulate those forming at the seasonal polar caps of Mars. The octagonal crystals measure 10 to 15 μm. 850X.*

In conclusion, our studies have attempted to illustrate that snow crystals and ice grains can be easily imaged by LTSEM. The collection procedures enable samples from distant locations to be frozen and shipped to a laboratory for storage and/or observation. Imaging identical samples, which are maintained in their frozen state, can also be done with a video or light microscope in any laboratory at ambient temperatures. Neither the expense nor the discomforts of a cold laboratory are necessary. Stereopsis (three-dimensional viewing) used in association with LTSEM greatly increases the ease of structural interpretations. The preparation procedures that are used for LTSEM do not result in sublimation or melting. Furthermore, the LTSEM increases the resolution and depth of focus attainable with a light microscope so that detailed surface structure not previously attainable can be imaged and recorded.

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