Collecting, Shipping, Storing, and Imaging Snow Crystals and Ice Grains With Low-Temperature Scanning Electron Microscopy

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ABSTRACT   Methods to collect, transport, and store samples of snow and ice have been developed that enable detailed observations of these samples with a technique known as low-temperature scanning electron microscopy (LTSEM). This technique increases the resolution and ease with which samples of snow and ice can be observed, studied, and photographed. Samples are easily collected in the field and have been shipped to the electron microscopy laboratory by common air carrier from distances as far as 5,000 miles. Delicate specimens of snow crystals and ice grains survive the shipment procedures and have been stored for as long as 3 years without undergoing any structural changes. The samples are not subjected to the melting or sublimation artifacts. LTSEM allows individual crystals to be observed for several hours with no detectable changes. Furthermore, the instrument permits recording of photographs containing the parallax information necessary for three-dimensional imaging of the true shapes of snowflakes, snow crystals, snow clusters, ice grains, and interspersed air spaces. This study presents detailed descriptions of the procedures that have been used successfully in the field and the laboratory to collect, ship, store, and image snow crystals and ice grains. Microsc. Res. Tech. 62:19–32, 2003.   Published 2003 Wiley-Liss, Inc.[†]

INTRODUCTION

Visual observations and descriptions of snow crystals began at least 2,000 years ago in ancient China when Han Ying indicated that "Flowers . . . of snow are always six pointed" (Hobbs, 1974). However, during the last hundred years, the use of light microscopy (LM) has enabled investigators to observe, photograph, describe, and classify numerous types of snow crystals and ice grains (Bentley, 1904, 1923; Bentley and Humphreys, 1931; Dobrowolski, 1903; Hallet, 1965; Hellman, 1893; Magono and Lee, 1966; Nakaya, 1954; Nordenskiöld, 1893). One of the most noteworthy attempts to illustrate natural snow crystals was undertaken by Wilson Bentley, a Vermont dairy farmer and amateur meteorologist. Bentley set up an outdoor laboratory and spent nearly 40 years photographing with the light microscope over 6,000 snow crystals mostly consisting of dendrites and plates (Blanchard, 1970). About 20 years later, Nakaya (1954) established a laboratory with a controlled environment to experimentally determine the effects of temperature on the formation and growth of all forms of snow crystals including dendrites, plates, columns, needles, and irregular crystals. However, the light microscope limited magnification of these forms to about 500×.

In spite of the vast knowledge that was obtained with the aid of the LM, limitations of the instrument and handling of the samples frequently compromised these studies. For example, poor depth of field prevented resolution of all but the very flat crystals, magnifications rarely exceeded 500×, melting and sublimation could easily occur during photomicrography, refraction and reflection of incident light confused internal structures with surface features, and adverse working conditions were frequently required in the laboratories. To eliminate some of these limitations, Kuroiwa (1969), Schaefer (1949), Stoyanova et al. (1987), and Takahashi and Fukuta (1987) attempted to make stable replicas that could be examined in an electron microscope. Use of the replicas further increased resolution of the surface of flat crystals; however, the ability to image intact specimens with three-dimensional topography remained elusive. Another approach was attempted by Cross (1969) who used a scanning electron microscope (SEM) to examine evaporating ice. However, because the sample was imaged in the vacuum of the instrument and not maintained at below freezing temperatures, sublimation and melting limited the observations. These problems were solved by equipping

the SEM with a cold stage that maintained the temperature of the ice sample near that of liquid nitrogen. As a result, samples of ice could be imaged, analyzed, and photographed (Barnes et al., 2001; Cullen and Baker, 2000, 2002; Cullen et al., 2002; Dominé et al., 2001; Iliescu et al., 2002; Kuroiwa, 1969; Muguruma, 1961; Mulvaney et al., 1988; Wolff et al., 1988).

Recently, studies of snow crystals have been undertaken with SEMs that were equipped with cold stages (Wergin and Erbe, 1994a–c; Wolff and Reid, 1994). Because the SEM has resolution and depth of field that exceeds those of the LM, this instrument provides detailed structural features of surfaces and allows three-dimensional imaging of the snow crystals. Furthermore, the cold stage prevents melting and sublimation; therefore, snow crystals and ice grains remain stable for indefinite periods of time. Using this technique, Wolff and Reid (1994) attempted to collect, ship, and image snow samples with a SEM. Snow was collected in Greenland, stored at liquid nitrogen temperature, and transported to England for observation. However, because of difficulties in collecting and mounting the samples, many were lost or broken during transport and the authors were only successful in imaging fragments of crystals that had been collected at the remote site. In the same year, Wergin and Erbe (1994a–c) also captured snow crystals near their laboratory, quickly transferred them to the SEM, and were able to observe numerous forms of intact crystals. Since that time, our laboratory has illustrated and described snow crystals and ice grains that have been collected from numerous remote locations (Foster et al., 1996; Rango et al., 1996a–c; 2000; Wergin et al., 1995a; 1996a,b; 1998a,b; 2002a,b). The purpose of this study is to provide detailed descriptions of the equipment and procedures that were fabricated and successfully used to collect, ship, store, and image snow and ice with low-temperature SEM (LTSEM).

MATERIALS AND METHODS

The equipment and techniques described below enable collecting, shipping, storing, and observing snow crystals and ice grains that are maintained at near liquid nitrogen temperatures ($-196°C$). At this temperature, specimens remain stable during observation in the LTSEM where they can be imaged and photographed. Many of the devices that are described are unique and must be fabricated in the laboratory because they are currently unavailable commercially. Manufacturers have been encouraged to produce many of the simple devices, which can be used for samples in a wide variety of scientific fields. The devices, which were originally designed for studies with LTSEM, can also be used for observations with light microscopy.

The term "cryo-system" is used generically in this study; it refers to an accessory that is available from several manufacturers and can be retrofitted to most SEMs. A cryo-system typically consists of: (1) a specimen holder (Fig. 1a); (2) a vacuum transfer assembly mechanism, consisting of a rod that attaches to the specimen holder and is used to move the holder through the various stages or compartments of the cryo-system; (3) a freezing chamber, where specimens are typically plunged into liquid nitrogen (LN_2) or some other cryogen; (4) a pre-chamber, where the specimen can be fractured and/or coated with a heavy metal and; (5) a cold stage, which is mounted in the microscope and can be maintained at near LN_2 temperatures.

Secondary Specimen Holders (Sampling Plates)

A commercial cryo-system normally comes with a single universal specimen holder; replacements or spare holders of this type frequently cost several hundred dollars each. When collecting snow or ice at remote sites, several dozen specimens are frequently desired. Therefore, numerous specimen holders are essential. To solve this problem, a simple, inexpensive secondary specimen holder or sampling plate was devised. The plate is used in the field to collect the sample. In the laboratory, the plate is accommodated by a modified commercial specimen holder, which is compatible with the cryo-system.

Our sampling plates are fashioned from sheets of stock copper, 1.5 mm thick, that are cut into individual pieces measuring 15 × 29 mm. One surface of the plate is uniquely numbered using a metal stamp for identification purposes; the opposite surface is roughened or textured to enhance adhesion of a cryo-adhesive and the specimen (Fig. 1b). Production of these plates in lots of 1,000 allows us to collect hundreds of samples at multiple sites, thereby supporting large-scale field experiments where snowpack properties are typically measured at vertical intervals of 10 cm.

The design of the commercial specimen holder was modified to accommodate our custom plates (Fig. 1c,d). The specimen holder, containing the plate, attaches to the transfer assembly mechanism and is inserted into the Oxford CT 1500 HF cryo-preparation chamber that is affixed to a Hitachi S-4100 SEM.

Storing Sampling Plates

Plates that contain frozen samples are stored in two different manners. Square brass tubing stock, 13 × 13 mm inside diameter, is cut into 20-cm lengths; a cap is permanently soldered on one end. At the other end, two holes are drilled in the opposing sides (Fig. 1e). Each tube is numbered for easy identification in subsequent steps. Under LN_2, a large forceps is used to insert six plates diagonally into the tubes; each plate is alternated by 90°. After the last plate is inserted, a paper clip is bent and inserted through the two pre-drilled holes to retain the plates in the tube during shipping and storage. For large samples, which may require more vertical clearance, square brass tubing stock, 22 × 22 mm inside diameter, is used and the specimen plates are inserted diagonally along their long axes.

At the collection site, tubes containing the sampling plates are lowered into a lightweight dry shipping Dewar or Cryopak Shipper (Taylor Wharton, Theodore, AL) that had been previously cooled with LN_2. The Dewar is placed in a backpack or hand carried from the collection site and then either transported by van 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 fully 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 stor-
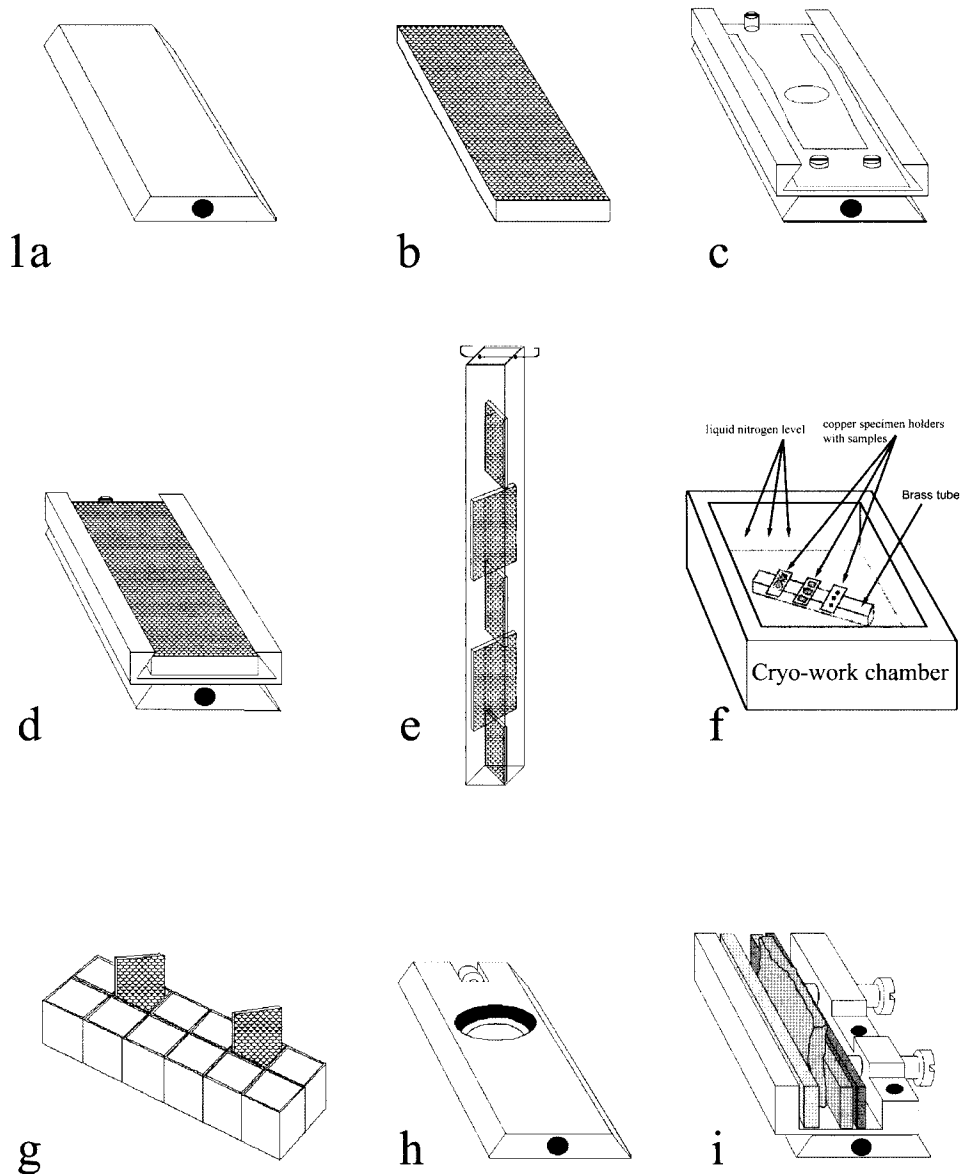
Fig. 1. Drawings of devices that were fabricated in the author's laboratory. **a:** Drawing of the standard specimen holder that is supplied with the Oxford CT 1500 HF Cryo-system. The rod of the specimen transfer device screws into the holder (black hole) and enables the specimen to be moved through the pre-chamber to the cold stage in the SEM. **b:** Drawing of a sampling plate, 15 × 29 mm, that is cut from stock sheet of copper, 1.5 mm thick. One surface of the plate is numbered for identification purposes; the opposite surface (shown in the diagram) is roughened to enhance adhesion of the frozen specimen. **c:** Drawing of a modified specimen carrier that is fabricated and attached to the standard specimen holder (lower level) depicted in a. **d:** Drawing of the modified specimen carrier containing the sampling plate depicted in b. The entire assembly, which attaches to the transfer rod of the cryo-system, can then be moved through the pre-chamber and onto the cold stage in the SEM. **e:** Drawing of the square brass tubing, 13 × 13 mm ID. The bottom end is permanently capped; two holes are drilled in the opposing sides of the upper end. Four plates can be inserted diagonally into the tubes; each plate is alternated at 90°. A paper clip inserted through the holes retains the plates in the tube during shipping and storage. **f:** Drawing of a Styrofoam work chamber, 12 × 20 × 4 cm (depth) where samples collected on the plates are either contact or plunge frozen in LN_2 and held until they are transferred to the square brass shipping tubes shown in e. A Styrofoam cover is used between sample collections to prevent condensation or frost from forming in the LN_2. **g:** Drawing of the staging jig that holds up to 10 plates in the Styrofoam box prior to loading in the square brass tubes. This device is fashioned by soldering together twelve 1-cm segments of the square brass tubing. **h:** Drawing of a standard cylindrical stub holder, which is used to hold a metal tube containing an ice core. The tube is placed in the circular well and held firmly by tightening the setscrew (far end). In the pre-chamber, the core can be fractured to expose an untouched, pristine, surface. **i:** Drawing of an indium vise specimen holder. An ice sample can be clamped into the holder, where it is held between two sheets of indium metal. Indium, which is thermally conductive and pliable at LN_2 temperatures, helps prevent shattering of the sample. This device has been used to hold glacial ice, icicles, hail, ice lenses, pond ice, and dry ice.

age Dewar where they remain until being further pre-
pared for observation with LTSEM.

Sampling Procedures

Preparation of Sampling Plates. All samples of
snow and ice are collected on the sampling plates,
which are pre-coated with a thin layer of a cryo-adhe-
sive such as Tissue Tek, a methyl cellulose solution.
The adhesive and the plates must be cooled to temper-
atures at or slightly below freezing. However, the ad-
hesive will solidify at temperatures below −3°C; there-
fore, it must be protected from freezing in the con-
tainer, as well as on the sampling plate prior to
sampling. To keep the adhesive near its freezing point
(−3°C), the stock bottle can be placed in a pocket of
one's parka. Similarly, cold plates, which will also
freeze the adhesive prior to sampling, must be main-
tained at near freezing temperatures. Immediately af-
ter a specimen has been collected, the plate containing
the adhesive and sample are plunged into a vessel of
LN_2. For this purpose we use a Styrofoam box, 12 ×
20 × 4 cm (depth) containing a 1- to 2-cm layer of LN_2.
The box is used to plunge freeze the plates, which
contain the samples, and to hold them for short-term
storage until they are transferred to the square brass
shipping tubes (Fig. 1f). A tight-fitting Styrofoam cover
or lid is used between manipulations to prevent exces-
sive condensation or frost from forming in the LN_2.
This container is extremely light, has low heat capacity
requiring very little LN_2 to cool, is inexpensive, and
easily replaced if damaged.

The Styrofoam box also contains a staging jig that
allows up to twelve plates to be frozen and stored prior
to loading them into the square brass channeling. This
device is fashioned by soldering twelve 1-cm segments
of the square brass channeling into two rows (Fig. 1g).
The loaded specimen plates fit into the jig diagonally
similarly to the manner in which they are loaded into
the shipping tubes.

In warm or sunny conditions, such as late spring or
on glacier surfaces during the summer, the tempera-
ture of the plates and the cryo-adhesive may be well
above freezing. When this occurs, the plates and the
adhesive can be pre-cooled by placing them in the
snowpack and then proceeding as described above. In
these cases, excellent preservation of structure has
been accomplished if the sampling is done rapidly and
the plate is immediately (less than one second) plunged
into LN_2. The possibility of melting the snow samples
at the point of contact with the adhesive is possible, but
does not affect the upper exposed surfaces of the spec-
imens.

Sampling Fresh and Falling Snow. Falling snow
is sampled by allowing it to settle on the surface of the
plate containing the cryo-adhesive. Alternatively, a
fresh sample can be lightly brushed onto the plate. In
either case, the plate containing the sample is then
rapidly plunge frozen in the LN_2 vessel.

When air currents are minimal, attempts to deter-
mine the orientation of descent of snow crystals, such
as those having accreted rime, are made by inverting a
plate, which contains the adhesive, and gently pressing
it onto the surface of the freshly fallen snow. When
observed in the LTSEM, this procedure exposes the
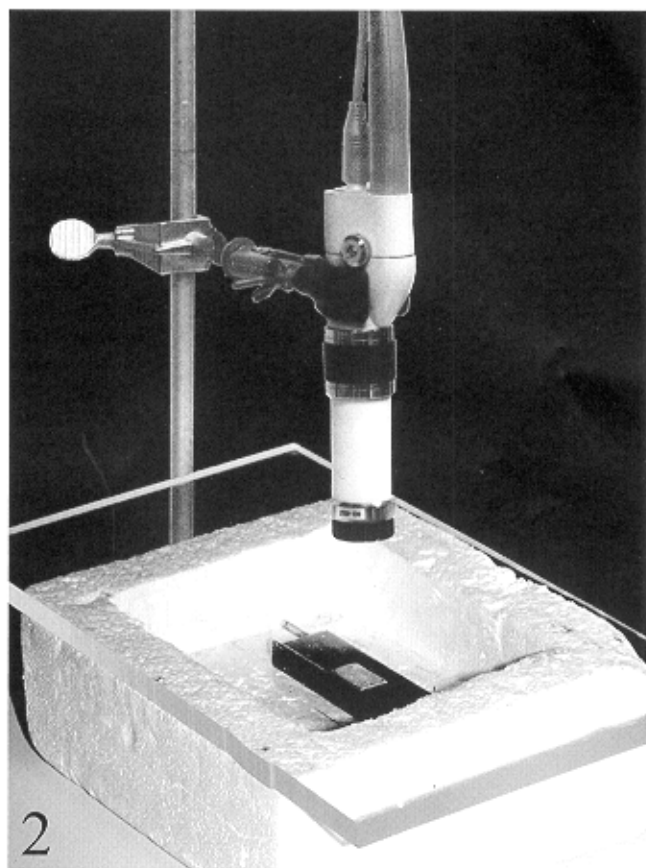leading surfaces of the crystals during descent. Alter-

Fig. 2. HIROX Hi-Scope KH-2200 Video Microscope System
equipped with an MX-250Z lens and extension tube mounted on a ring
stand above the Styrofoam box shown in Figure 1f. The box is covered
with a 12-mm-thick transparent sheet of lexan, which helps to reduce
surface condensation and fogging. The video microscope is interfaced
to a monitor and a computer that is used to acquire, display, or store
digitized images of the sample.

natively, allowing the crystals to settle on the plate
reveals their trailing surfaces when imaged in the in-
strument. Under minimum air movement, the majority
of crystals will maintain their fall orientation, which
can be determined in this manner.

Snowpit Samples. To collect samples from snow-
pits, a pre-cooled (LN_2) scalpel is used to gently dis-
lodge snow crystals from a freshly excavated pit wall.
The crystals are allowed to accumulate onto a plate
containing the cryo-adhesive and then plunged rapidly
into the LN_2. If LN_2 cannot be carried to the snowpit, a
brass block, pre-cooled with LN_2, is transported to the
pit. After the crystals are sampled, the plate is placed
on the brass block and allowed to freeze, then processed
as described above.

Collection of sintered snow crystals, which is more
difficult, requires breaking and removing a cluster of
crystals from their native site. Classically, investiga-
tors placed a cluster of sintered snow crystals on the
surface of a ruled measuring card. Then the sample
is broken so that the individual crystals and the
markings on the card can be observed. The crystals
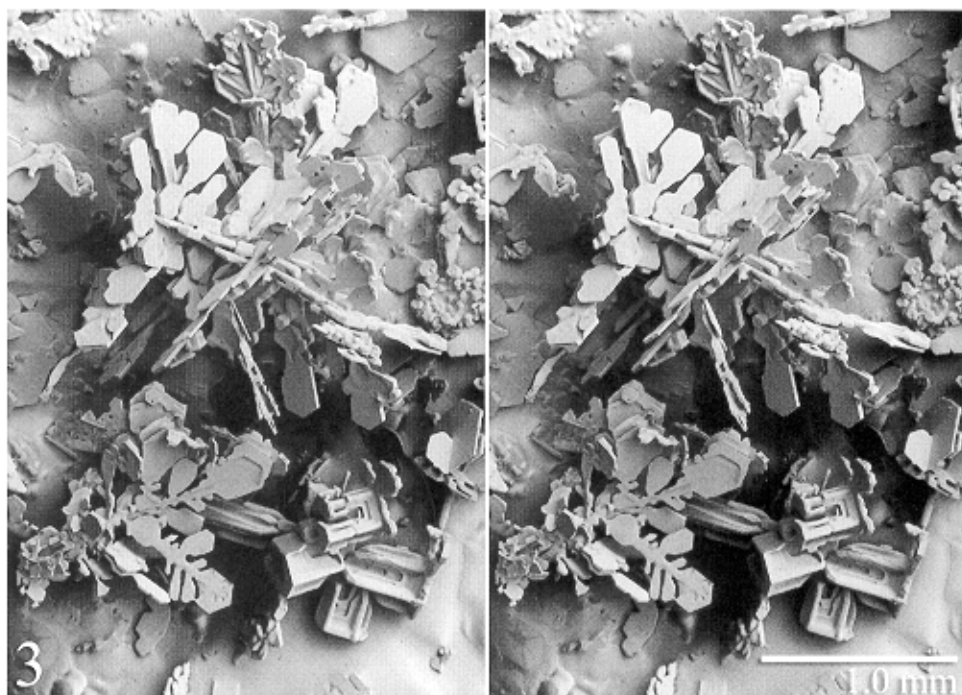are observed with a hand lens and notations are

Fig. 3. Stereo pair illustrating snowflakes that consist of capped bullets (lower left), dendrites and hexagonal plates. These crystalline aggregates probably result from atmospheric "collisions" during descent. Sample collected from West Virginia.
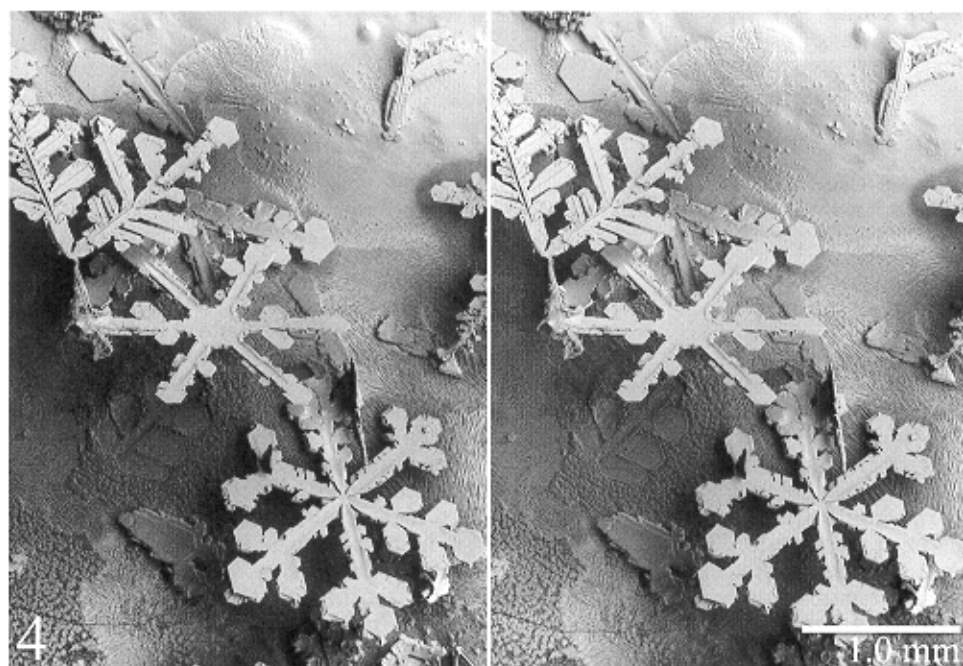

Fig. 4. Stereo pair illustrating two intact hexagonal dendrites that were collected by exposing a specimen plate, which was covered with cryo-adhesive, to falling snow. The delicate arms of the two dendrites are intact and do not exhibit any damage resulting from collecting, freezing, storing or transporting the sample. Sample collected in West Virginia and transported by vehicle in a dry shipper at $-196°C$.

made concerning the size and degree of faceting that exists. To sample analogous crystals for imaging with the LTSEM, 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. Alternatively, if air volumes and sintering are to be examined, an entire cluster of sintered crystals can be dissected from the pit wall, mounted intact on the plate and frozen.

Similarly, this technique can be used to sample wind slab, sun crust, or surface hoar, which may exist in the stratified layers of the snowpit.

Hoar Frost. Plates can also be placed in an area where rime, surface hoar, or frost are expected to occur and simply allowed to serve as the substrate for the sample that forms. In this case, the cryo-adhesive is not necessary. When a sufficient sample has sublimed on the plate, it is plunge frozen in LN_2.
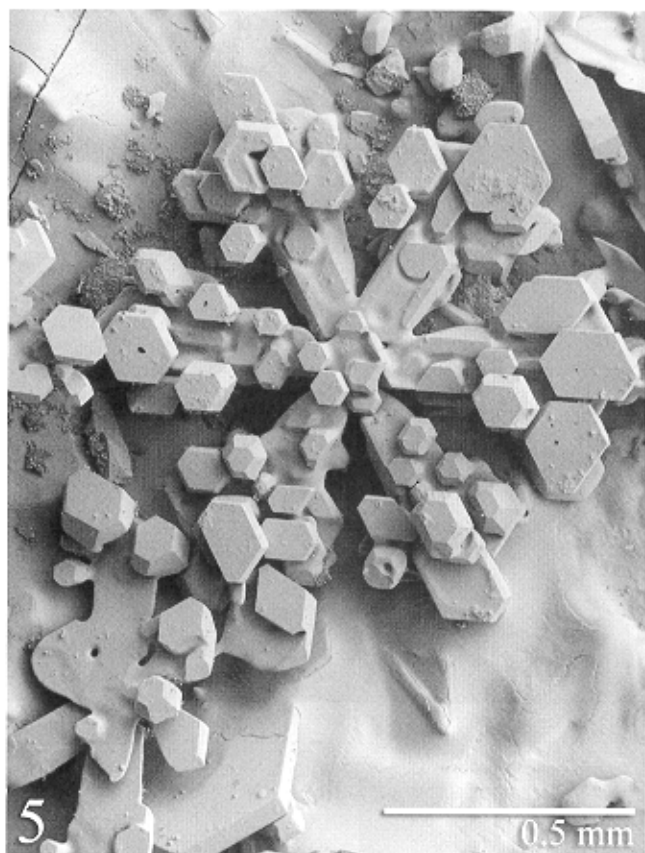
Fig. 5. Hexagonal dendrite containing numerous irregular snow crystals, in the form of small hexagonal plates, on its surface. This crystal was collected by gently pressing a specimen plate to the surface of freshly fallen snow. As a result, the face that was imaged represents the leading surface of the snow crystal. Sample collected in Wisconsin and shipped to laboratory by priority air express.
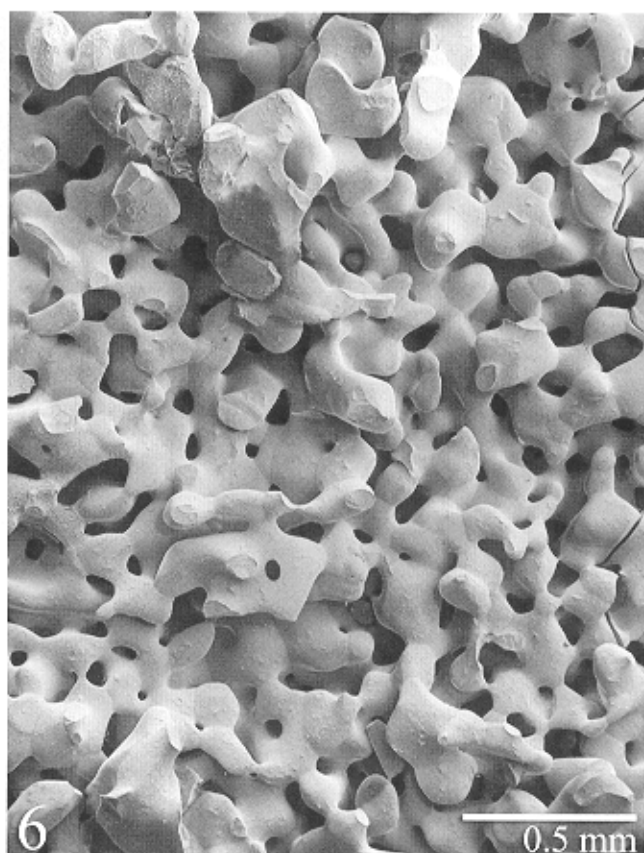
Fig. 6. Sample of an icy wind crust consisting of a variety of highly sintered and metamorphosed snow crystals. Sharp edges or facets that characterize the freshly falling snow crystals are no longer apparent. Sample collected and shipped from Alaska.

Fractured Samples. Fracturing of snow clusters or glacial ice is used to reveal the extent of air spaces, details of sintering and biota, such as ice worms, snow algae (*Chlamydomonas nivalis*), fungi, and bacteria. This process is accomplished with a pick that is mounted in the pre-chamber of the cryo-system. Prior to coating, the pick is used to randomly fracture or remove a portion of the snow cluster or ice sample. This process exposes a pristine internal surface that is then coated and inserted into the LTSEM for imaging.

Glacial Ice. Glacier ice is normally sampled with a coring tool. Smaller samples for LTSEM are collected with a secondary ice corer consisting of a modified brass cork borer in which teeth are filed to aid in cutting. The borer is pre-cooled in LN_2 and a small core, 5 to 8 mm in length, is removed with the cork borer, placed into LN_2, stored, and shipped to the laboratory in a brass tube. At the laboratory, portions of the core are placed into a cylindrical specimen holder (Fig. 1h) containing the cryo-adhesive. At this time, the frozen core can be fractured under LN_2 to expose untouched, pristine, surfaces of the glacial ice.

Alternatively, a slab of ice can be removed, using a small pre-cooled saw. At the laboratory, a portion of the slab is clamped into a modified vise holder where the sample is held between two thin sheets of indium metal that is thermally conductive, allows containment and helps prevent shattering (Fig. 1j). This device has also been used to attach portions of icicles, hail, ice lenses, pond ice, and dry ice (CO_2).

Artificial Snow. Another sample variation involves the study of artificial snow. Plates coated with the cryo-adhesive, are merely placed in the plume of artificial snow that is being produced by a snow gun. After a suitable amount of sample has accumulated on the plate, it is plunge frozen in LN_2.

Carbon Dioxide Crystals. Plates or holders are inserted into the pre-chamber of the cryo-system and cooled to near LN_2 temperatures. Carbon dioxide gas is introduced into the pre-chamber and allowed to sublime onto the plates. This procedure can also be used to produce crystals of other gases that can be examined with the LTSEM.

Coating Samples

All frozen samples are coated with 2 to 10 nm of platinum using a magnetron sputter coating device in a high purity argon environment within the pre-chamber. This process makes the samples electrically conductive and enhances secondary electron emission for
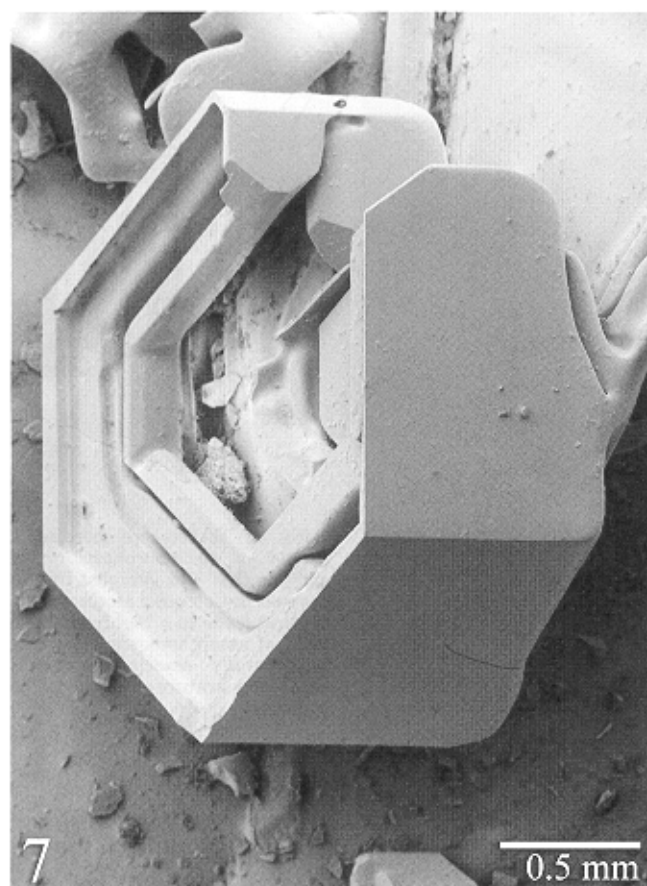
Fig. 7. Early stage of depth hoar crystal obtained from a freshly excavated snowpit in northern Minnesota. This type of crystal, which is associated with a large temperature gradient in the snowpack, has a smooth faceted outer surface and a complex internal structure. Depth hoar, which is not sintered, can become several millimeters in size.

imaging in the LTSEM. The argon gas, which is introduced during this process, must be very free of water vapor and other impurities that could contaminate the surface of the specimen. During coating and imaging, any area of the specimen that possesses poor thermal conductivity will etch or sublimate and the continuity of the overlying coating will be compromised. As a result, the sample will be poorly coated and tend to charge.

Recording Images With an SEM

All specimens were imaged with 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 was maintained at −130° to 185°C. Accelerating voltages of 500V to 10 kV were used to observe the samples. However, 2 kV was most commonly used because it minimized charging and provided adequate resolution. The samples were imaged for as long as 2 hours without observing any changes in the structural features or in the coating integrity of the snow crystals. Selected images were recorded onto Polaroid Type 55 P/N film (Polaroid, Cambridge, MA).

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

Recording Images With a LM

Samples collected, shipped, and stored on the copper plates are not only prepared for LTSEM observation, but the same plates can be observed and photographed at atmospheric pressure using a light microscope (video imaging). The video imaging is done in the laboratory through a 1.5-cm-thick transparent, lexan plastic cover that is placed on the open Styrofoam box previously described. Before the samples are placed in the box, the following procedures are taken to prevent melting or sublimation of the sample. A block of aluminum, 3.5 × 12 × 1 cm (mass = 137 g) is placed in the bottom of the box and covered with LN_2. The box is covered with the lexan and the block is allowed to cool. When the temperature has equilibrated, a previously stored plate containing a sample is transferred to the surface of the aluminum block in the Styrofoam box. The level of the LN_2 is adjusted so that the sample is exposed above the liquid and the lexan cover is placed over the box. At this time, the aluminum block remains submerged in the LN_2; the sample is in an atmosphere of cooled N_2 gas and remains close to the LN_2 temperature due to thermal conduction through the plate.

To photograph the sample, a HIROX Hi-Scope KH-2200 Video Microscope System is interfaced to a video monitor and computer, which acquires, displays, and stores digitized images of the sample. The scope is equipped with a MX-250Z lens and an extension tube that results in a working distance of 20 cm and a magnification of 50×. The Hi-Scope is mounted vertically above the box with a bracket on a ring stand (Fig. 2). Illumination is provided either with a fiber optic system that is built into the lens or by fiber optic or incandescent side lighting. Additional magnifications and working distances can be obtained by substituting other Hi-Scope lenses or by inserting the Hi-Scope into the camera tube of a Wild Makroskop M420.

To obtain stereo pairs of photomicrographs, the entire Styrofoam box, or the specimen plate, is gently tilted 6–10° after the first image is recorded. The two images contain the parallax information necessary for stereopsis or three-dimensional viewing. After the video images are obtained, the plate containing the sample can be attached under LN_2 to the cryo-system specimen holder and transferred to the pre-chamber of the cryo-system for coating and observation in the LT-SEM.

RESULTS
Fresh Falling Snow

A snowflake is " . . . an assemblage of individual snow crystals which have collided and remained fastened together during their fall through the atmosphere" (LaChapelle, 1969). The assemblages that we have examined frequently contain several different forms of crystals, which may include the basic types such as dendrites, plates, columns, needles, and irregular crystals, as well as variations of these types. For
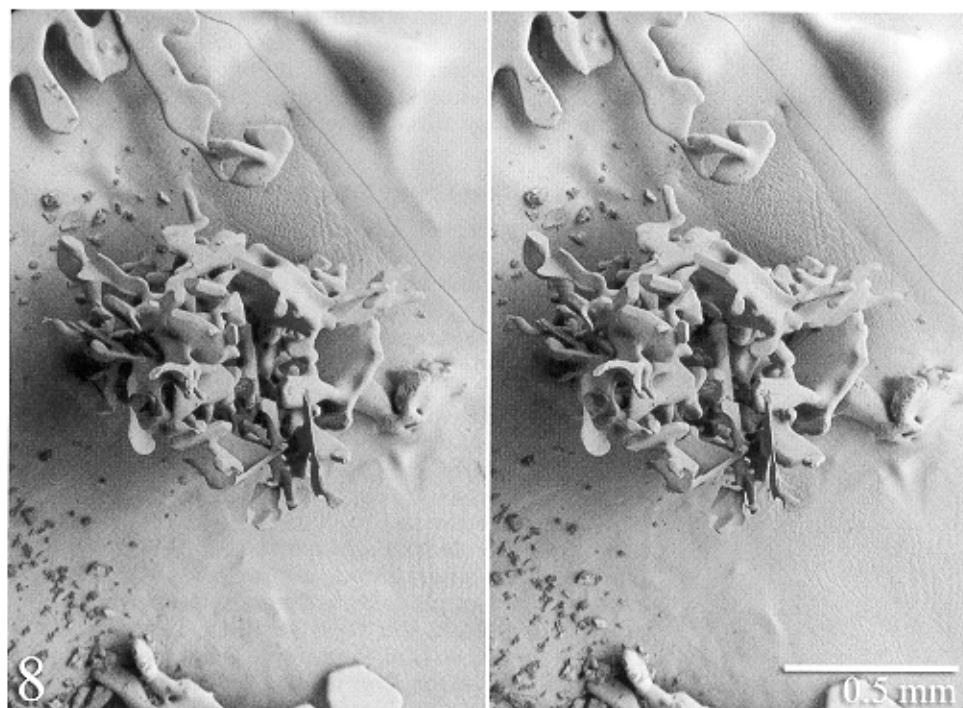
Fig. 8. Stereo pair showing a cluster of metamorphosed snow crystals that was sampled from a snowpit in Colorado. Metamorphosis that leads to a highly sintered layer of amorphous snow crystals is generally associated with a small temperature gradient in the snowpack.

example, Figure 3 illustrates snowflakes that consist of capped bullets, dendrites, and hexagonal plates. Fragments of crystals are also commonly encountered in the samples; whether they result from damage during collection and shipping or from atmospheric collisions cannot be determined. However, intact hexagonal dendrites are frequently found in samples that were collected and shipped from remote sites, indicating that delicate snow crystals are usually undamaged by these procedures (Fig. 4).

Figure 4 illustrates a sample of freshly falling snow that was collected by allowing the crystals to fall and accumulate on the surface of a plate, which was covered with the cryo-adhesive (Fig. 4). In Figure 5, a plate, containing the adhesive, was gently pressed to the surface of the newly fallen snow (Fig. 5). In the latter case, if the crystals were not subjected to wind currents, the leading surface of the snow crystal will be exposed and imaged when the sample is observed in the LTSEM. In this case, a dendrite covered with small irregular crystals, in the form of small hexagonal plates, represents the leading surface.

Snowpit Samples

An established snowpack reveals a variety of metamorphosed crystals resulting from wind, melting, and temperature gradients. Windy conditions can result in a layer commonly known as an icy wind crust that consists of highly metamorphosed and sintered crystals (Fig. 6). These crystals, which do not exhibit the sharp edges or facets that characterize the freshly falling snow, are typically rounded and smaller.

Digging a snowpit and exposing its internal face provides examples of the metamorphosed crystals that result from temperature gradients that exist in the snowpack. Large temperature gradients gradually result in the formation of large weakly sintered crystals commonly referred to as depth hoar (Fig. 7). These crystals typically exhibit a complex multi-layered structure consisting of steps that are apparent externally, as well as internally. Alternatively, small temperature gradients result in a highly sintered layer of amorphous snow crystals (Fig. 8). Individual snow crystals (Fig. 7) or clusters of snow crystals (Fig. 8) can be successfully removed from the face of the snowpit for preservation and study.

Sintered Snow/Fractured Samples

Another type of well-sintered snow crystals occurs in spring when daytime temperatures are above freezing but nighttime temperatures drop below 0°C. Under these conditions, partial melting of the snow grains produces a film of free water that surrounds the individual crystals and moves downward through the snowpack. Samples that are removed from the snowpack and frozen in LN_2 exhibit remnants of snow crystals, which appear as spherical grains 0.3 to 0.6 mm in diameter. The grains are engulfed in a smooth amorphous matrix consisting of the free water that froze at the time of plunge freezing in LN_2 (Fig. 9).

"Red" Snow

Occasionally, the snowpack 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. The fracturing process, which was previously described, can be used to reveal the algae that populate the water film (Fig. 10). The algae, which are unicellu-
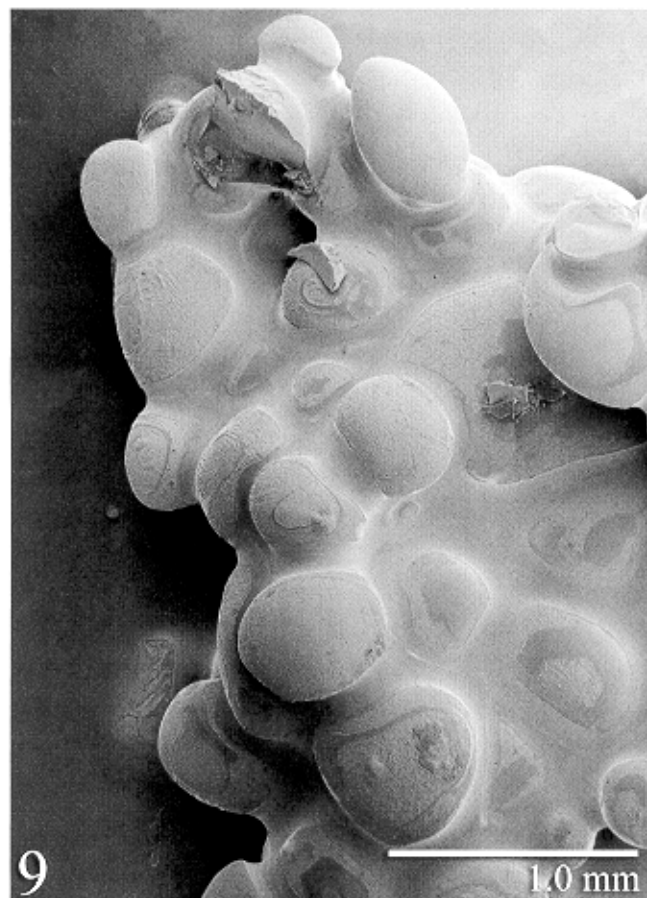
Fig. 9. Metamorphosed crystals that result from freeze/melt conditions in late spring or summer. Under these conditions, partial melting of the snow grains produces a film of free water that surrounds the individual crystals, which appear as spherical grains, 0.3 to 0.6 mm in diameter. Sample was collected in July at Loveland Pass, Colorado, 11,800 feet above sea level.
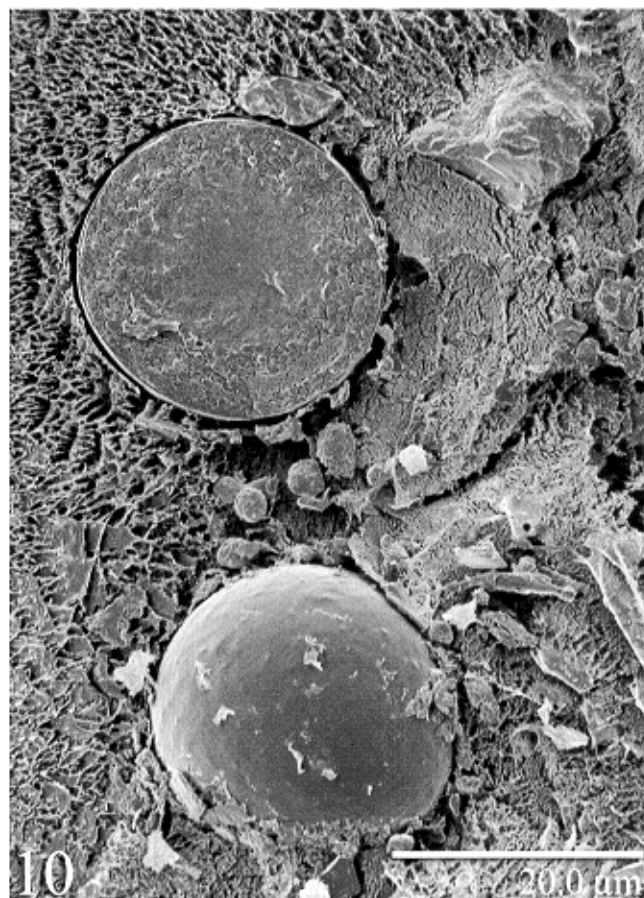
Fig. 10. "Red" snow is frequently associated with prolonged freeze/melt conditions. The term results from the reddish hue that is associated with a pigmented alga, *Chlamydomonas nivalis*, that is found in the water film of the melting snowpack. Fracturing a snow sample reveals unicellular algae, 20 μm in diameter. An intact, spherical alga cell and a fractured, circular cell are present in the top and bottom micrograph, respectively. Sample was collected in July at Loveland Pass, Colorado, 11,800 above sea level.

lar and circular in fractured samples, measure approximately 20 μm in diameter.

Hoar Frost

In addition to fresh and metamorphosed snow, LT-SEM is used to image frost or surface hoar. When night temperatures fall below the frost point, snow crystals or microscopic ice grains preferentially form or sublimate on surfaces that contain nucleating agents. Hoarfrost will form on organic as well as on inorganic surfaces. Consequently, the frost can be collected either directly on a sampling plate or by transferring an object containing the frost, such as a pine needle, to a plate. Figure 11 illustrates a dense formation of irregular crystals that formed on surface of a blade of grass.

Ski Tracks

The mere weight of a skier on newly fallen snow compresses the snowpack and breaks the snow crystals (Fig. 12). When the skier glides across the surface of this snow, the crystals in the resulting ski track are markedly changed (Fig. 13). Portions of broken crystals

now appear surrounded by a smooth film of ice, similar to that found surrounding snow grains under freeze/melt conditions. Perhaps the friction from the ski causes some surface melting that results in a film of water. Subsequently, this water film re-freezes either from the low ambient temperature of the snowpack or from plunging the collected sample in LN$_2$.

Glacial Ice

In addition to snow crystals, ice cores from glaciers were imaged with the LTSEM. The surface layer of ice, which represent early firn formation, is composed of large, irregularly shaped ice grains, about 1 mm in diameter, that are sintered with adjacent grains (Fig. 14). Air spaces between the ice grains are prevalent at this stage. Older cores are composed of a solid layer of ice. Fractures, which reveal a pristine inner surface of the ice sample, exhibit numerous circular air pockets, which represent gas volumes within the ice core (Fig. 15). The pockets, which are 15 to 25 μm in diameter, are not interconnected with one another but exist as discrete and faceted voids surrounded by the glacial ice.

Fig. 11.   Surface hoar or frost, consisting of a dense aggregation of irregular crystals and a few short columns that formed by vapor deposition on the surface of a blade of grass. Frost samples forming on biological or non-biological substrates can be mounted on specimen plates and frozen in LN_2 to preserve their structural integrity. Sample collected in West Virginia.

Fig. 12.   Surface of a fresh snowpack that was compressed by the weight of a cross-country ski. Crystals are compacted and broken. Sample collected in West Virginia.

Artificial Snow

Natural snow generally forms when a nucleating particle triggers vapor deposition; a solid forms directly from the vapor or gas molecules. The result is a snow particle 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 contains ice nucleating particles, is atomized under pressure and sprayed into a cold atmosphere, where the droplets quickly freeze. As a result, artificial snow appears as relatively large frozen water droplets that do not possess any of the morphological attributes of natural snow (Fig. 16). The size of the droplets, which can vary from 0.1 to 1.0 mm, is a function of the pressure and type of gun that is used to atomize the water.

Carbon Dioxide Crystals

Crystals of gases other than water vapor can also be imaged with LTSEM. Figure 17 illustrates crystals of CO_2 gas that was allowed to enter the pre-cooled prechamber and condense on a sample plate. The condensate reveals octahedron crystals that measure 10 to 15 μm. These crystals of CO_2 frost are believed to be similar to those that comprise, along with water ice, the seasonal polar caps of Mars (Leighton and Murray, 1966).

LM/SEM Comparisons

Photomicrographs of crystals obtained with a video light microscope can be somewhat ambiguous because the image is formed by light that is transmitted, reflected, and refracted by the crystal (Fig. 18). Consequently, details of the external features of a crystal may be masked by internal features. LTSEM can be used to observe a unique crystal after it has been observed and photographed with the LM (Fig. 19). With LTSEM, only the external features of the crystal contribute to the final image. The structural details, which are clear and unambiguous, represent the true surface features of the crystal.

DISCUSSION

Although LTSEM has only recently been used to study snow crystals, this is not a new technique. Echlin et al. (1970) originally described the technique to image frozen, hydrated specimens of biological tissue. Since that time, LTSEM has been used to observe numerous types of biological tissues and to resolve macromolecu-

Fig. 13. Ski track in snow described above that resulted from the pressure and friction of a cross-country ski moving over the surface of freshly fallen snow. The sample consists of crushed snow crystals that are embedded in a smooth layer of ice. This ice may form from water that resulted from heat and friction generated by the ski. Sample collected in West Virginia.

Fig. 14. Sample from the "dirty ice layer," South Cascade Glacier, Washington. The ice grains on the surface of the old glacier are large, irregularly shaped, and sintered. Prevalent air spaces are found among the ice grains at this stage. Curved surface of the coring is evident at the top.

lar structure (see Wergin et al., 1999b). Similarly, cryo-fixation and coating, which consist of plunging a sample into a cryogen, and coating with a heavy metal, are not new procedures. Nearly 50 years ago, Steere (1957) demonstrated the use of these techniques to prepare replicas of virus particles for observation in a transmission electron microscope. Through the years, this technique has also been widely applied to biological studies.

During the last 50 years, investigators (Kuroiwa, 1969; Muguruma, 1961; Truby, 1955) have used modifications of these procedures to create and examine replicas of ice in an electron microscope. The LTSEM techniques used in the current study merely combine the cryo-fixation and coating steps from the earlier established procedures. However, rather than observing a replica, the stage of the SEM is maintained at near LN_2 temperatures permitting the actual samples of frozen snow and ice to be observed.

In response to previous studies conducted in our laboratory, reviewers have suggested that the use of LN_2 to freeze, store, and ship samples may be too harsh or extreme and that imaging with LTSEM may alter structure of the sample. However, LN_2, which has extremely low surface tension, exerts minimal force on the frozen surface of the sample. This is not only evidenced by the preservation of delicate specimens of snow crystals, such as those shown in Figure 4, but has been demonstrated in extremely fragile biological samples including bacteria, fungi, nematodes, plants, insects, and mites (Wergin et al., 1998b, 1999b, 2000). In these cases, delicate unicellular structures are preserved as well as, tenuous phoretic and parasitic interactions between organisms.

Liquid nitrogen is also widely used to store bacteria, fungal spores, plant seeds, and nematodes. In these cases, not only is the structural integrity of the samples maintained, but they also retain their viability and will germinate and grow when properly thawed. Likewise, animal semen, which is used for artificial insemination, is stored in LN_2. Because LN_2 has no apparent adverse effects on the structure or physiology of these biological samples, some of which contain 85 to 95% water, we believe that samples of snow and ice are likewise unaffected. Furthermore, during storing and shipping of the samples, the preservation of the delicate structure of crystals may also be enhanced by the fact that water ice increases in hardness as the temperature is cooled to that of LN_2.

To further document that coating and imaging samples with LTSEM do not affect the structure, individual
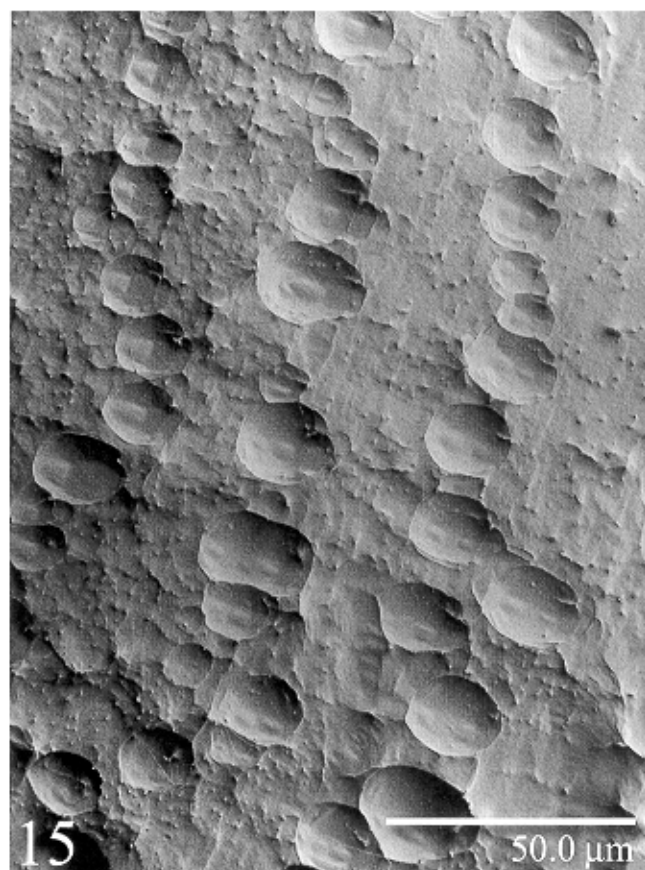
Fig. 15.   Sample of ice core taken at South Cascade Glacier, Washington. Fractured interior of the ice coring illustrates the presence of numerous faceted air pockets, 15 to 25 μm in diameter, that remain in the core at this stage.

Fig. 16.   Newly formed artificial snow collected from a plume generated by a snow gun in Vermont. Artificial snow forms when water, generally at temperatures less than −3°C, is atomized under pressure. The minute droplets, which quickly freeze before reaching the ground, can vary in size from 0.1 to 1.0 mm as a function of the pressure and type of gun that was used to atomize the water.

snow crystals and ice grains were photographed using video light microscopy at −196°C degrees, transferred to the pre-chamber of the cryo-system, platinum coated, and then re-photographed in the LTSEM. Comparisons of the images are shown in Figures 18 and 19. In previous studies, samples were removed from the LTSEM and photographed a third time by returning to the video light microscope (Rango et al., 1998; Wergin et al., 1998a,b). Comparisons of the first video images with the second video images, which were recorded after coating and observation in the LTSEM, failed to reveal any structural changes. As a result, we suggest that procedures described in this study for sampling, shipping, storing, and imaging snow crystals and ice grains have no detectable structural effects or alterations on specimens that are properly handled.

The procedures described in this study have been used to increase our understanding of fresh and metamorphosed snow as well as glacial ice and CO_2 ice. LTSEM examination of fresh snow provides images with detailed structure of plates, dendrites, needles and columns (Rango et al., 1996a; Wergin et al., 1994a,b; 1995a,b; 1996a,b). In addition, the resolution of this technique allows characterization of irregular crystals (Wergin et al., 2002a,b). This form of snow

crystal was initially described nearly 50 years ago (Nakaya, 1954), and is recognized in the International Commission on Snow and Ice (Colbeck et al., 1990). However, LM is unable to resolve their detailed structure. Likewise, LTSEM easily resolves the frozen cloud droplets that collect on snow crystals. The technique allows detailed descriptions of the accretion of the droplets on snow crystals, a process that results in the formation of rime and graupel (Rango et al., 2003; Wergin et al., 1999a).

The techniques described in this study are particularly useful for collecting and examining metamorphosed samples of snow crystals. Classical techniques frequently used a hand lens, under adverse conditions, to characterize the snow crystals that are sampled from snowpits. The procedures described in this study allow sampling and characterization of numerous specimens from snowpits at multiple sites (Rango et al., 1996b,c; Wergin et al. 1995a, 1996b). Furthermore, the samples can be fractured to study and identify biota that may be present in the late spring and summer snows (Rango et al., 2000; Wergin et al., 1996b).
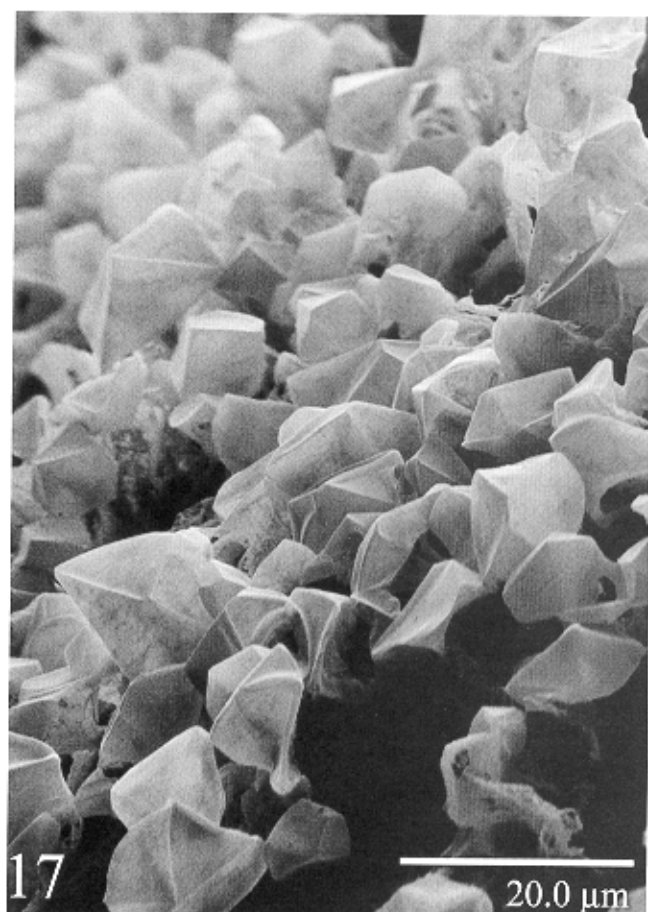
Fig. 17. Crystals of CO_2 that may simulate those forming at the seasonal polar caps of Mars. The crystals were produced by allowing CO_2 gas to condense on a specimen plate in the pre-chamber, which was held at the atmospheric pressure of Mars, of the cryo-system. The octahedron crystals measure 10 to 15 µm. Crystals of other exotic gases might also be observed by this technique.

LM photomicrographs of ice samples are frequently difficult to interpret because the image is formed by light that is reflected and refracted from the external as well as internal surfaces of the sample. Alternatively, the LTSEM image is formed only from the outer surface that is exposed to the electron beam. Consequently, the technique clarifies the structure of individual grains in firn and glacial ice and easily resolves grain boundaries, interconnecting air spaces, and the microscopic air pockets that exist in glacial ice and icicles. (Rango et al., 2000; Wergin et al., 1996c).

The ability to tilt the stage in a LTSEM permits samples to be observed and recorded at different angles. Two micrographs, differing by a 6–10° tilt angle, contain the parallax information that is necessary to view the samples in three-dimension (Rango et al. 1996c; Wergin et al., 1995b). This feature, combined with the depth of field available in the LTSEM, allows imaging of all samples of snow and ice that exhibit topography well beyond the focal plane of a LM.

Recently, investigators have equipped LTSEMs with energy dispersive X-ray microanalysis systems. The combination of these two techniques not only enables them to characterize the structural features of a sample but also permits them to identify the elemental constituents and contaminants that may be present. These techniques have been used to analyze snowflakes (Wolff and Reid, 1994), but have been more extensively applied to identifying impurities and their distribution in natural ices (Cullen et al., 2002; Iliescu et al., 2002), including Vostok ice (Cullen and Baker, 2002) and polar ice (Barnes et al., 2001; Cullen and Baker, 2000; Mulvaney et al., 1988; Wolff et al., 1988). Future research in this area may provide valuable information on the type and source of pollutants that are captured during snow and ice formation, as well as on how the elemental composition of polar and glacial ice changes through the ages.
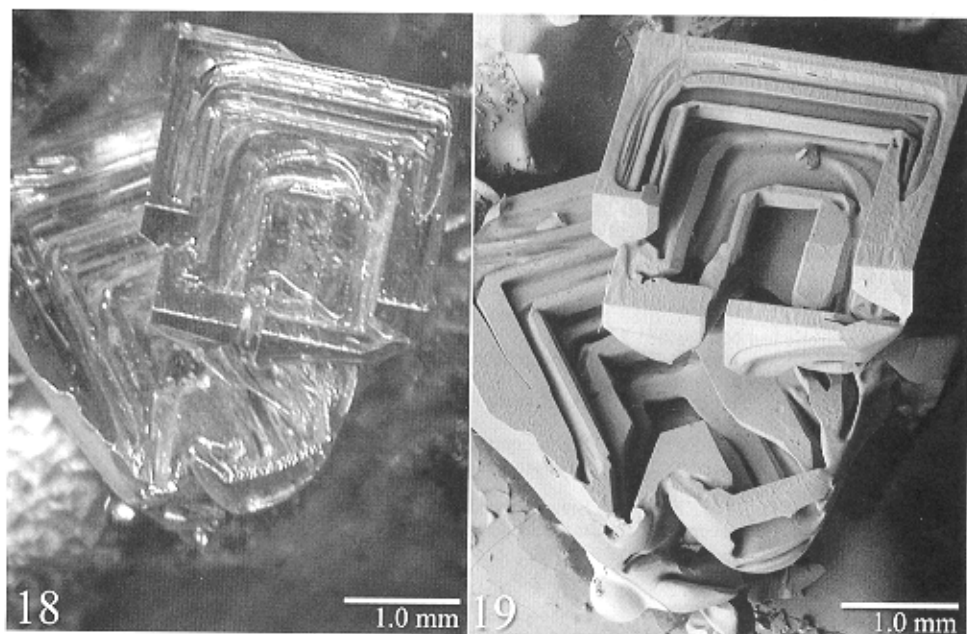
Figs. 18–19. Example of a depth hoar crystal collected from the base of a snowpit in Wyoming. Comparison of video and LTSEM images of the identical crystal illustrates that the faceting and internal features in the video image are somewhat compromised by the transmitted, reflected, and refracted light that occurs with LM. The external features are more distinct in the LTSEM image.

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