JMSA ISSN: 1079-8501

Vol. 2, No. 3: pp. 99–112, 1996

# Low Temperature SEM of Precipitated and Metamorphosed Snow Crystals Collected and Transported from Remote Sites

**William P. Wergin,**[1*] **Albert Rango,**[2] **Eric F. Erbe,**[1] **and Charles A. Murphy**[1]

*Nematology Laboratory*[1] *and Hydrology Laboratory,*[2] *Agricultural Research Service,*
*U.S. Department of Agriculture, Beltsville, MD 20705*

**Abstract**: Procedures were developed to sample, store, ship, and process precipitated and metamorphosed snow crystals, collectively known as "snowflakes," from remote sites to a laboratory where they could be observed and photographed using low temperature scanning electron microscopy (LTSEM). Snow samples were collected during 1994–96 from West Virginia, Colorado, and Alaska and sent to Beltsville, Maryland for observation. The samples consisted of freshly precipitated snowflakes as well as snow that was collected from pits that were excavated in winter snowfields measuring up to 1.5 m in depth. The snow crystals were mounted onto copper plates, plunged into $lN_2$ and then transferred to a storage dewar that was shipped to the laboratory. Observations, which could be easily recorded in stereo format (three-dimension), revealed detailed surface features on the precipitated crystals consisting of rime, graupel, and skeletal features. Samples from snowpacks preserved the metamorphosed crystals, which had unique structural features and bonding patterns resulting from temperature and vapor pressure gradients. In late spring, the surface of a snowpack in an alpine region exhibited a reddish hue. Undisturbed surfaces from these snowpacks could be sampled to observe the snow crystals as well as the organisms responsible for the coloration. Etching the surface of samples from these sites exposed the presence of numerous cells believed to be algae. The results of this study indicate that LTSEM can be used to provide detailed information about the surface features of precipitated and metamorphosed snow crystals sampled at remote locations. The technique can also be used to increase our understanding about the ecology of snow. The results have application to research activities that attempt to forecast the quantity of water in the winter snowpack and the amount that will ultimately reach reservoirs and be available for agriculture and hydroelectric power.

**Key words:** Low temperature SEM, snowflake, snow crystal, freeze-substitution, algae.

(received March 28, 1996; accepted June 12, 1996)

## 1. INTRODUCTION

About a hundred years ago, the combination of light microscopy with photography allowed investigators to begin to magnify and record the variations that existed in the structure of snow crystals (Nordenskiold, 1893; Hellman, 1893; Dobrowolski, 1903). In spite of the numerous problems associated with working with frozen microscopic particles at subzero temperatures, an amateur microscopist named Wilson A. Bentley spent nearly 30 years amassing over 6,000 photomicrographs of snow crystals (Blanchard, 1970). However, because of his apparent interest in symmetrical forms, Bentley concentrated on the flat or two dimensional crystals, namely plates and dendrites, which could be properly focused and photographed with transmitted light in the relatively narrow depth of field of his light microscope. Although over 2,000 of these photomicrographs were eventually published (Bentley and Humphreys, 1931), he did not include the atmospheric conditions under which the snow was collected or the magnifications at which the crystals were photographed. Furthermore, because the publication concentrated on symmetrical crystals rather than on the full range of shapes that Bentley surely must have encountered, it is somewhat compromised in its scientific value.

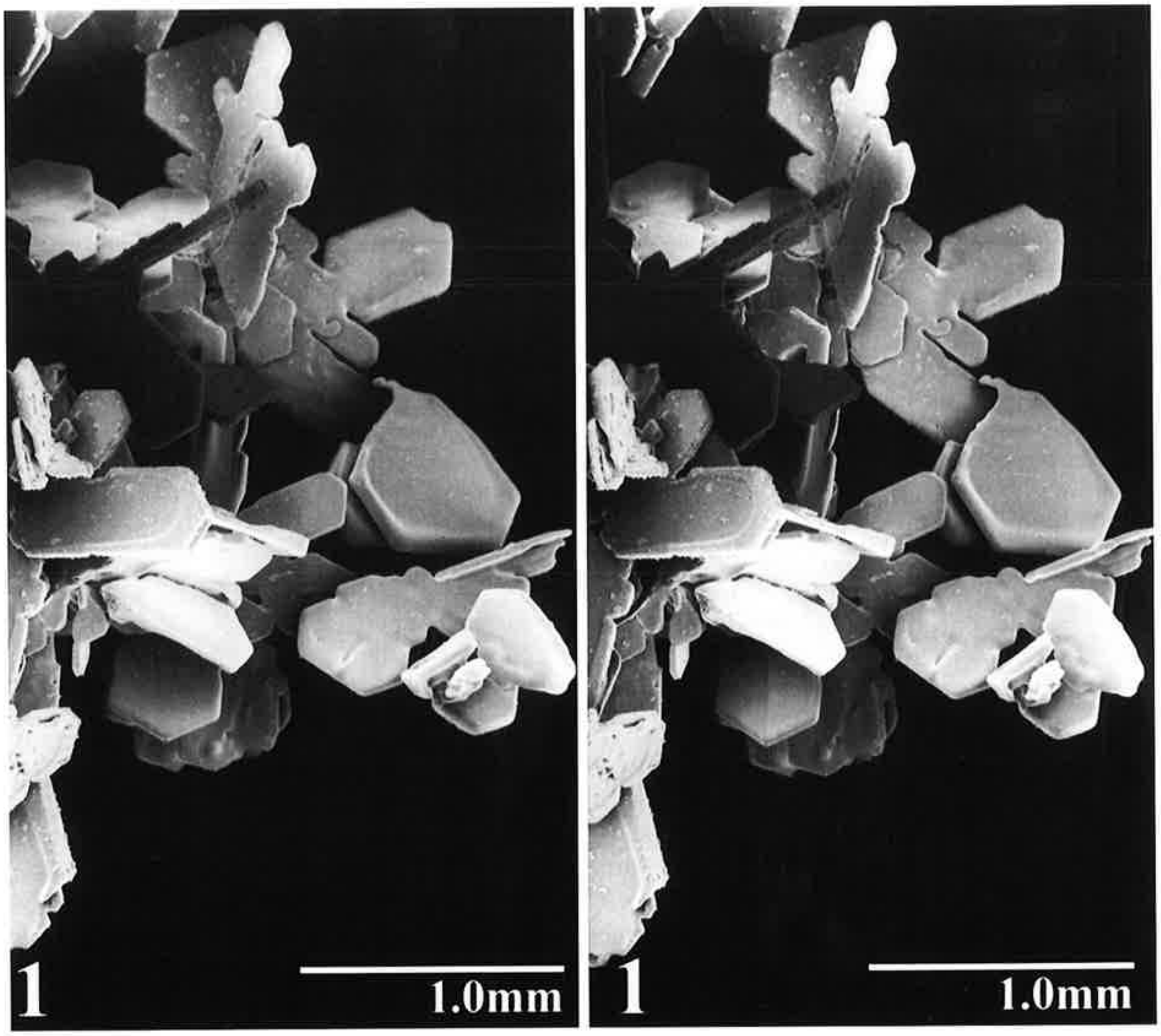
These deficiencies were remedied by Nakaya, who

---

*Corresponding author: Electron Microscopy Unit, Bldg. 177B, USDA-ARS, Beltsville, MD 20705.
Phone: (301) 504-9027, fax: (301) 504-8923, e-mail: WWERGIN@GGPL.ARSUSDA.GOV

began a light microscopic investigation in 1932 that lasted nearly 20 years (Nakaya, 1954). In his studies, oblique illumination was used to reveal the shapes and surface features of all types of snow crystals, including many three-dimensional forms such as needles and columns. Nakaya not only recorded photomicrographs of the structural variations of crystals that were collected from natural snows (during which he carefully recorded environmental conditions), but, under controlled laboratory situations, he was also able to simulate various atmospheric conditions to produce artificial snow crystals that resembled those from nature. Unfortunately, the photomicrographs of the three-dimensional types of snow crystals were greatly compromised by the limited depth of field in the light microscope, the structural details of rime and graupel could not be resolved, and other features such as the existence of double sheets and various skeletal patterns were represented with line drawings because they could not be convincingly recorded and illustrated in the photomicrographs.

To avoid the adverse working conditions that were necessary for light microscopic studies, and to increase the resolution of snow crystals, transmission electron microscopists attempted to make replicas of snow crystals that could be examined in the TEM. Use of replicas further resolved the details about the flat surfaces of two-dimensional crystals (Stoyanova et al.,



**Figures 1–3.** Snow samples collected at Beltsville, Maryland, elevation 60 m above sea level (ASL).

**Figure 1.** Stereo pair illustrating a snowflake composed of numerous snow crystals that consist mostly of irregular hexagonal plates. Sampled at 0°C air temperature.

1987; Takahashi and Fukuta, 1987); however, the ability to image intact, three-dimensional snow crystals remained elusive. Recently, Wergin and coworkers (Wergin and Erbe, 1994a; 1994b; 1994c; Wergin et al., 1995a; 1995b) used an SEM equipped with a cold stage to image various forms of newly precipitated snow crystals that were collected outdoors at their laboratory site. Their collection and processing procedures provided the first clearly focused images of intact snow crystals. In addition, this technique was used to record stereo images of single snow crystals as well as their aggregates, which are more commonly known as snowflakes (Wergin et al., 1995b).

During this same time period, Wolff and Reid (1994) made an attempt to collect, ship, and image newly precipitated snow crystals from remote sites. Snow samples were collected in Greenland, stored in liquid nitrogen, and transported to England for observation. However, because of difficulties in permanently mounting the samples, most of the specimens were either lost or broken during transport and the authors were only successful in imaging fragments of snow crystals that had been collected at the remote site. Because snow scientists (including glaciologists, geophysicists, and ice physicists) are frequently interested in the metamorphism of snow crystals that occurs in remote winter snowpacks, and because of our previous success in collecting and imaging newly precipitated snow crystals at our laboratory site, we were encouraged to develop procedures to collect, store, and ship newly precipitated as well as metamorphosed snow crystals from distant sites. The results of these efforts are presented in the current study.

## 2. MATERIALS AND METHODS

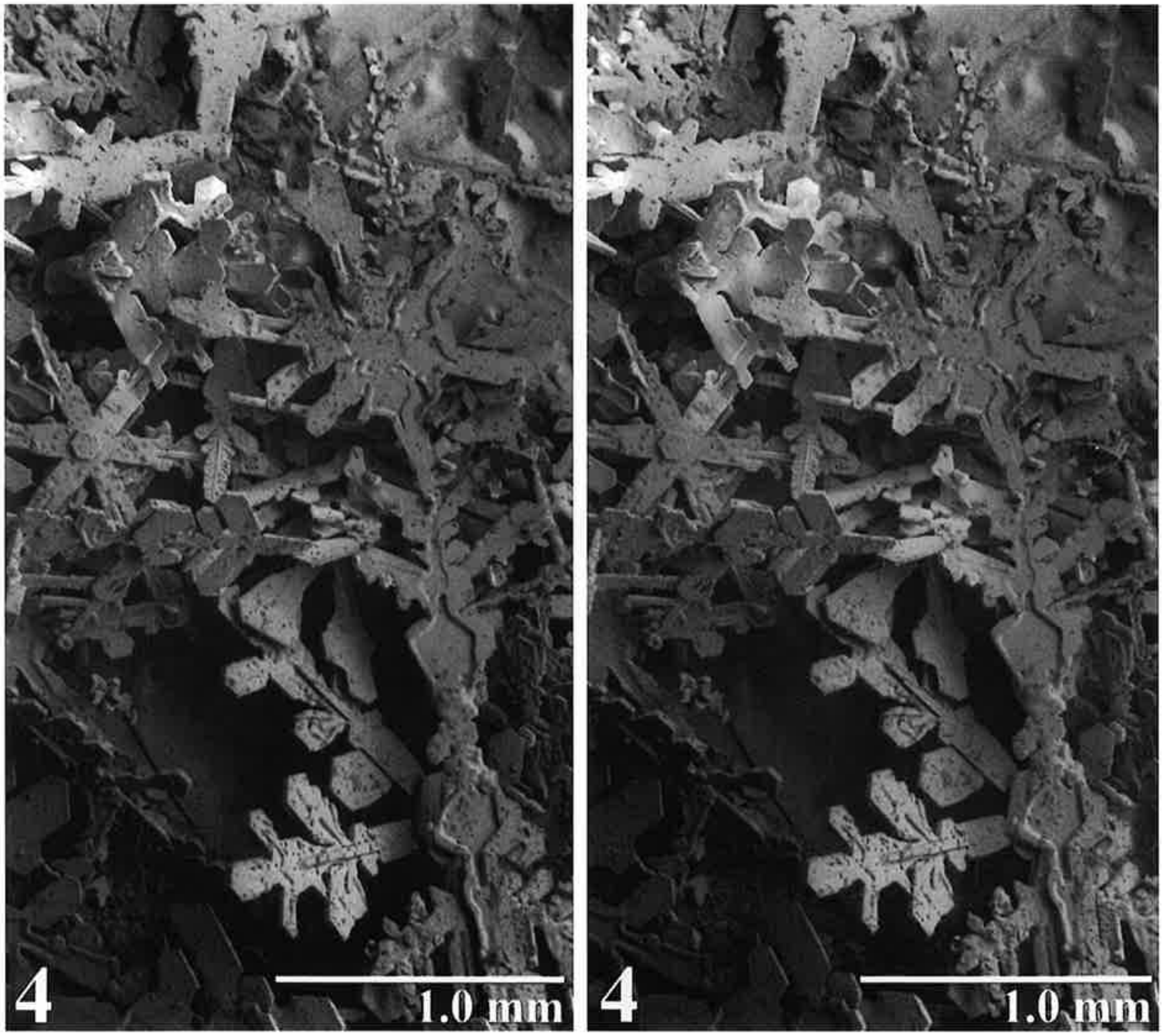
Snow was collected during 1994–96 from sites near the following locations: Beltsville, Maryland; Davis, West Virginia; Loveland Pass and Jones Pass, Colorado; and Fairbanks, Alaska. The samples, which were obtained when the air temperatures ranged from +18°C to -11°C, consisted of freshly fallen snowflakes as well as snow crystals that were collected from the walls of snowpits

**Figure 2.** Snowflakes consisting of crystalline needles encumbered with aggregations of short prismatic crystals. Sampled at -5°C air temperature.

**Figure 3.** Dendritic form of snow crystal lacking sharp edges. Because of the air temperature at the time of collection, this crystal may have undergone some sublimation or melting. Sampled at 0°C air temperature.

excavated in winter snowpacks measuring up to 1.5 m in depth. The snowpits were established in pristine areas that were located from about 50 m to nearly 10 km from plowed roadways. Sleds, snowshoes, crosscountry skis, snowmobiles, and a snow cat were used to transport the sampling supplies, including liquid nitrogen, to these remote sites.

The collection procedure consisted of placing a thin layer of liquid Tissue-Tek®, a commonly used cryo-adhesive for biological samples, on a flat copper plate (15 mm x 27 mm). The Tissue-Tek and the plates were precooled to ambient outdoor temperatures before use. Newly fallen snowflakes were either allowed to settle on the surface of the Tissue-Tek or were lightly dislodged and allowed to fall onto its surface. Next, the plate was either rapidly plunged into a styrofoam vessel containing liquid nitrogen ($lN_2$) or placed on a brass block that had been precooled with $lN_2$ to -196°C. This process, which solidified the Tissue-Tek, resulted in the firm attachment of the sample to the plate. When samples were obtained from snowpits, a precooled scalpel was used to gently dislodge crystals from the pit wall onto plates that contained Tissue-Tek; then the plates were rapidly plunged in $lN_2$. The frozen plates were inserted diagonally into 20 cm segments of square brass channelling and lowered into a dry shipping dewar that had been previously cooled with $lN_2$. The dewar containing the samples was conveyed from the



**Figures 4–8.** Samples collected on Bearden Mountain near Davis, West Virginia, elevation 1150 m ASL.

**Figure 4.** Stereo pair illustrating a snowflake consisting of dendritic snow crystals. Sampled at -11°C air temperature.

snowpit sites and then either transported by van (from West Virginia) or shipped by air (from Colorado and Alaska) to the laboratory in Beltsville, Maryland. Upon reaching the laboratory, the samples were transferred under $lN_2$ to a $lN_2$ storage dewar where they remained for as long as nine months before being further prepared for observation with LTSEM.

### Preparation for LTSEM Examination

To prepare the samples for LTSEM observation, the brass channelling was extracted from the storage dewar and placed in a styrofoam work chamber that was filled with $lN_2$. A plate was removed from the channelling and attached to a modified Oxford specimen holder. The holder, which contained the plate, was transferred to the slush chamber of an Oxford CT 1500 HF Cryotrans system that had been filled with $lN_2$. Next, the holder was attached to the transfer rod of the Oxford cryosystem, moved under vacuum into the prechamber for etching and/or sputter coating with Pt or Au/Pd, and then inserted into a Hitachi S-4100 field emission SEM that was equipped with a cold stage maintained at -185°C. Accelerating voltages of 500 V to 10 kV were used to observe and record images onto Polaroid Type 55 P/N film. To obtain stereo pairs, a stage tilt of 6° was introduced between the first and second images.

### Preparation for TEM Observation

After snow samples containing the alga cells were observed and photographed in the LTSEM, the specimen holders were withdrawn into the prechamber, removed, and then transferred under vacuum to $lN_2$ to begin a freeze-substitution procedure. The flat plates on which the samples were mounted were then transferred to 45 ml cryogenic vials that were filled with a solution of 2% (w/v) osmium tetroxide in acetone precooled to -196°C. The vials were placed in wells that had been drilled in an aluminum block. The aluminum block was put into a precooled brass chamber and then placed into an insulated encasement that had been precooled with $lN_2$. The insulated encasement was filled with dry ice to maintain a temperature of -80°C. The temperature of the samples was monitored with a thermocouple that was placed in a vial in the center well of the aluminum block. Substitution with the osmium solution was allowed to proceed for 3 days. Subsequently, the solution was slowly warmed (2 h at -60°C, 2 h at -18°C, 2 h at 4°C and 2 h at room temperature) and the substitution medium was replaced with fresh

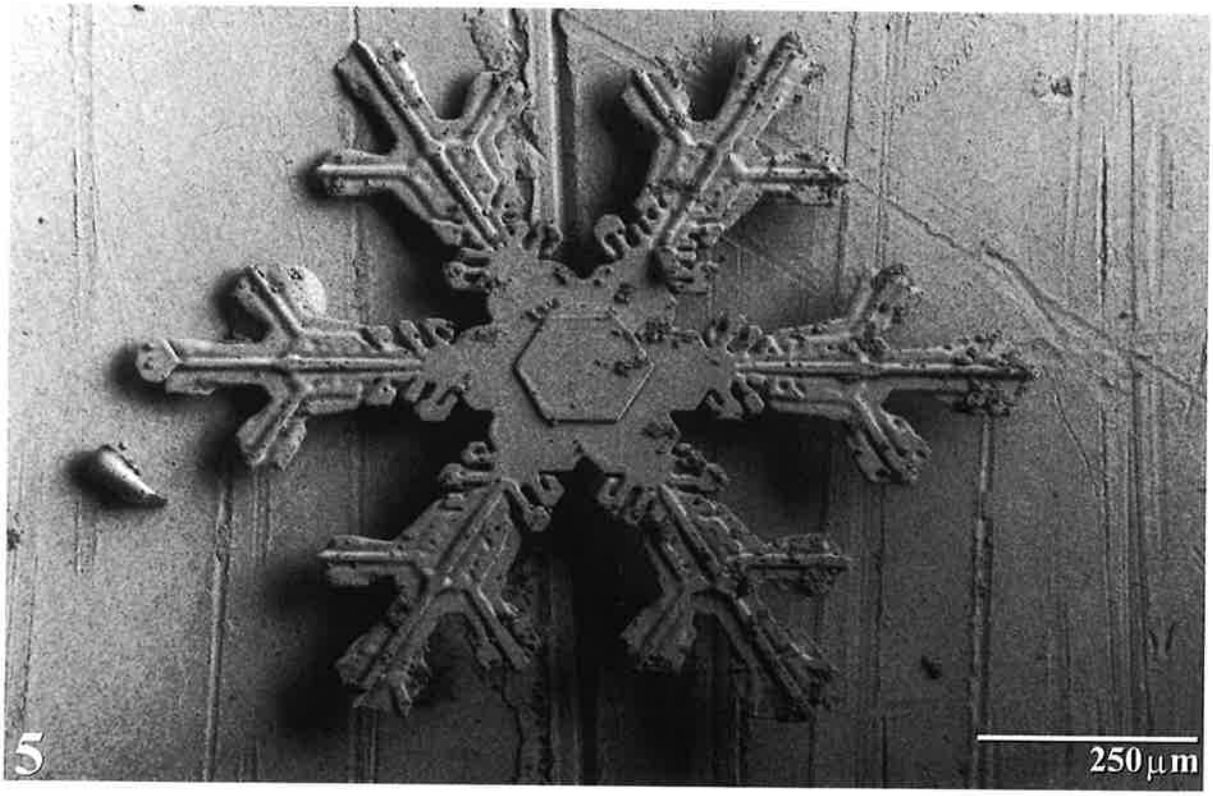


**Figure 5.** A hexagonal dendrite having a central hexagonal plate. The arms of the dendrite are branched and contain pronounced ridges and small depressions. Sampled at -11°C air temperature.

acetone. Next, the samples were detached from the plates, gradually infiltrated with Spurr's low viscosity resin, and cured at 60°C. The embedded samples were thin sectioned on an American Optical Ultracut ultramicrotome and mounted on copper grids. To improve contrast, sections were post-stained in 2% aqueous uranyl acetate and Reynold's lead citrate. TEM observations were performed on a Hitachi H500-H operating at 75 kV.

## 3. RESULTS

### Snowflakes — Aggregations of Snow Crystals

A snowflake consists of one or more snow crystals that either simultaneously grow together or make contact and become associated as they move through the atmosphere. The snow crystal aggregates that form snowflakes occur as variations of plates, needles, columns, or dendrites. The specific shape that prevails at a given time is largely dependent upon the cloud temperature at which the crystal forms (Hobbs, 1974). Snowflakes that largely consisted of aggregated plates (Figure 1) and needles (Figure 2) were collected outside the laboratory at Beltsville, Maryland. These samples were not stored or transported prior to observation at low temperature in the SEM.

The snowflakes, which were sampled when the air temperature was 0°C, measured 1 to 5 mm across and were composed of randomly aggregated variations of flat hexagonal plates (Figure 1). The individual plates varied from 0.4 to 0.8 mm across. Snowflakes that were collected at -5°C were generally smaller and consisted of bundles of needles that were encumbered with short prismatic crystals (Figure 2). The needles were 1.0 to 2.0 mm in length whereas the diameter of the bundles was about 0.2 to 0.3 mm; the short prismatic crystals that were present on the surfaces of the bundles measured about 0.1 mm.

Dendritic forms of snow crystals were only occasionally found in the Beltsville samples (Figure 3). This type of crystal was easily distinguished by its six arms, which were frequently branched. The structural similarity that often existed between the six arms of the dendritic forms provided hexagonal symmetry that characterized this type of crystal. The dendrites collected at Beltsville generally lacked the sharp edges that charac-

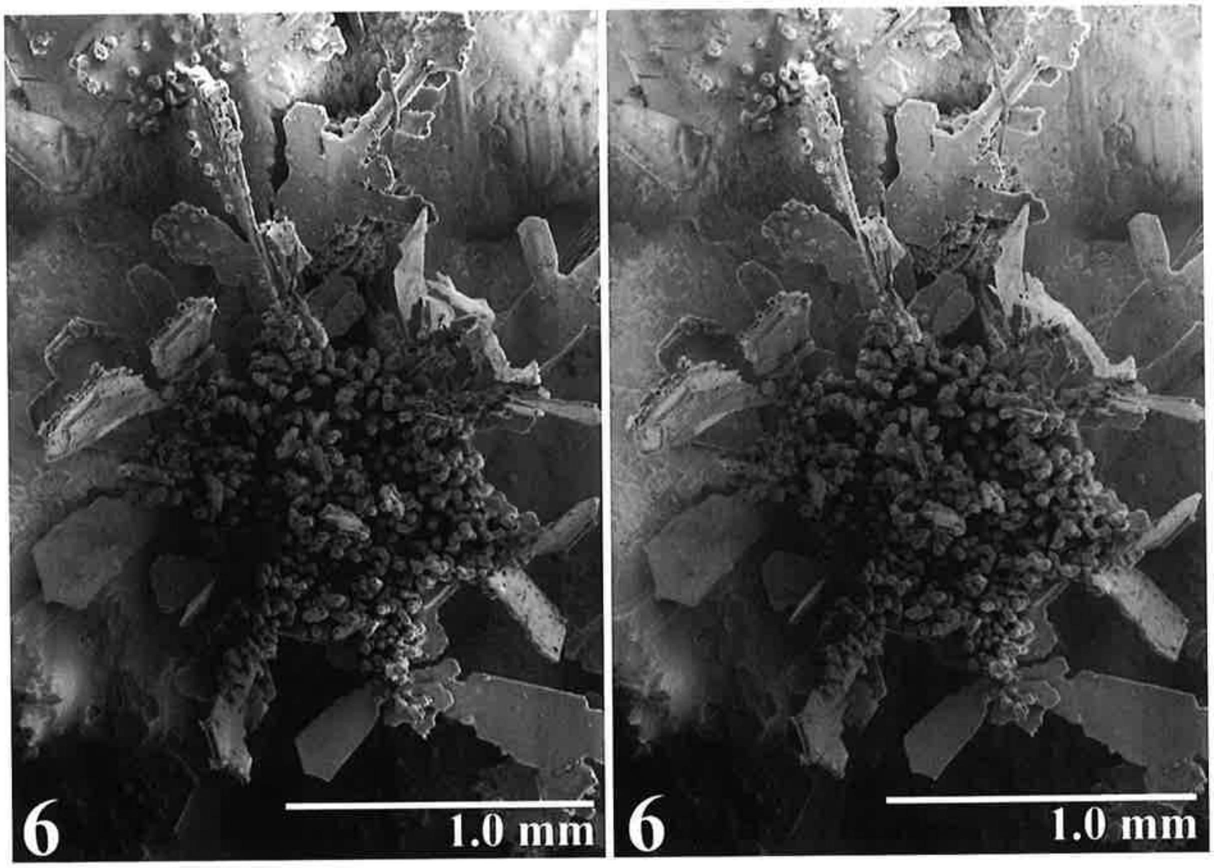


**Figure 6.** Stereo pair illustrating a snowflake that has become encumbered with supercooled water droplets or rime. Sampled at -2°C air temperature.

terized many of the other types of crystals. Furthermore, the dendritic arms generally exhibited less branching and similarity to one another compared to those from dendrites obtained from colder sampling sites.

Samples of snowflakes that were obtained from West Virginia were plunged in $lN_2$ at the collection site and transported by van to the laboratory in Beltsville, MD for storage and observation. At -11°C, a snowflake sample obtained from West Virginia was composed of overlapped and interlocked dendritic snow crystals (Figure 4). Although these crystals appeared more fragile than the hexagonal plates or the needles, neither the collection procedure nor transport in the dewars seemed to result in any significant breakage or damage. Although snow crystals with broken arms were occasionally observed, this type of damage could also have resulted from collisions with other crystals as they descended through the atmosphere.

Different forms of growth were frequently combined into a single snow crystal. In Figure 5, the hexagonal dendrite is the largest and most predominant form of snow crystal. However, closer examination revealed the presence of a small, flat hexagonal plate in the center of the dendrite. This dendrite also exhibited other distinct structural features that were frequently present on this form of snow crystal. Arms were often branched; in this crystal, a left and right pair of branches emanated from the main axis of each arm about two thirds from its base. A slightly raised and continuous midrib formed along the center of the arms and extended into the branches. Lateral to the midrib were a series of depressions, consisting of elongate channels and circular cavities. These depressions were believed to correspond to negative crystals in ice that have been previously described (Hobbs, 1974). The depressions frequently exhibited bilateral arrangement along the axis of the arms and branches. Because the occurrence and arrangement of ribs and depressions were fairly consistent in each of the six arms and their branches, these structural features reinforced the impression of hexagonal symmetry that was characteristic of this type of crystal.

## Further Resolution of Surface Features

Although numerous light micrographs of snow crystals have been recorded, clear illustrations of additional structural features such as rime deposits and microcrystalline growth are beyond the resolution of that technique. During formation and descent, snow crystals frequently encounter supercooled water droplets that freeze onto their surfaces. These particles did not exhibit crystallographic features, but alternatively appeared as random accumulations of small spheres measuring 0.02 to 0.06 mm in diameter (Figure 6). These frozen droplets are referred to as rime. When the droplets continue to accumulate until the original crystal is no longer distinguishable, the mass of randomly arranged, frozen microdroplets is referred to as graupel (Brownscombe and Hallet, 1967).

**Figure 7.** Dendritic crystal with central hexagonal plate and arms terminating with platelike sectors. Small raised hexagonal crystalline particles can be found on the surfaces of these terminal plates. Sampled at -8°C air temperature.

LTSEM also resolved the multiple layering and microcrystalline growth that occurred in many of the snow crystals (Figures 7 and 8). A hexagonal dendrite observed with the light microscope is generally thought of as a single crystal. However, a distinct, raised hexagonal plate was also frequently incorporated into this type of crystal (Figures 5 and 7). The plate was centrally located and positioned so that its six apices were aligned with the arms of the dendrite. In addition to the central plate, the platelike extensions at the ends of the arms contained raised particles. Unlike the rime, which tended to be spherical, these particles consisted of microcrystalline hexagonal structures measuring 0.04 to 0.06 mm. Even closer examination

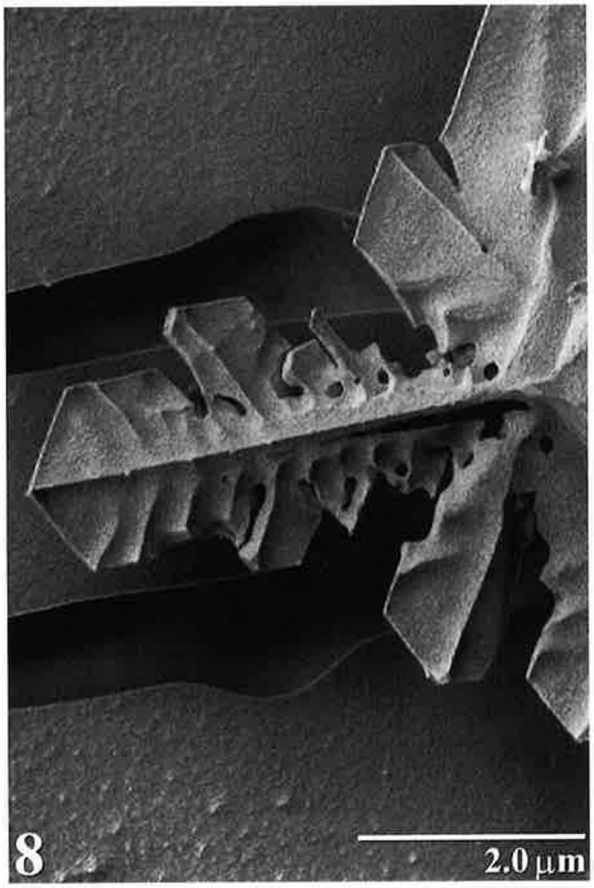


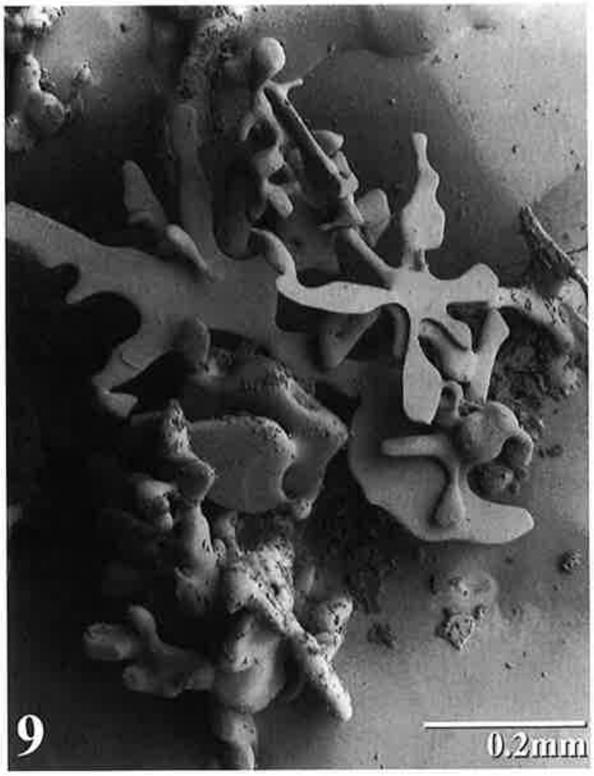
**Figure 8.** Apex of the hexagonal plate, delineated in Figure 7, illustrating a developing microcrystalline extension.

of the edges of a crystal revealed microcrystalline formations that probably contributed to further growth and layering along the arms of the dendrite (Figure 8).

### Metamorphism

In Colorado and Alaska, snow samples were taken from the accumulated winter snowpack to illustrate the types of transformations or metamorphism that occurred after the snow crystals reached the ground and were subjected to microenvironmental variations in temperature and vapor pressure. Snow crystals that were sampled several cm below the surface of snowpacks, which were not subjected to significant temperature differences between the ground and the air, underwent changes that are referred to as low temperature metamorphism (Figures 9 and 10). The initial changes were characterized by sublimation of the fine delicate structures on the edges and surfaces of the snow crystals (Figure 9). The sharp surface angles that characterized the crystalline features of a snow crystal were lost. As this process proceeded, the surface of the snow crystals became smooth and sinuous. In addition, adjacent crystals bonded or sintered to one another. At lower depths, where this process had proceeded for longer times, the original forms of the crystals were no longer discernable; they appeared rounded and well-bonded to one another, resulting in compaction of the snowpack and somewhat reducing the air space that had been present between the crystals (Figure 10).

A second type of metamorphism occurred in the snowpack when the temperature of the ground was significantly greater than the air temperature at the surface of the snowpack, i. e., when a temperature gradient of at least 10°C/m had been present. In this situation, high temperature gradient metamorphism results from a heat flux moving upward through the snow pack (McClung and Schaerer, 1993). Sublimation



**Figures 9–16.** Samples from either Colorado or Alaska, shipped by air to the laboratory and stored in $lN_2$ prior to imaging.

**Figure 9.** Snow crystals subjected to a low temperature gradient. The fine delicate features on the surface of snow crystals are no longer apparent. The crystals exhibit smooth sinuous contours and have become bonded with one another. The sample was collected from a snowpit 5 cm below the surface near Jones Pass, Colorado, elevation 3150 m ASL, air temperature -6°C.

occurred on the lower surfaces of crystals followed by recrystallization of the water vapor on upper-lying surfaces (Figure 11). With time, this process resulted in the formation and growth of large crystals known as depth hoar, found near the base of the snowpack (Figure 12). The crystals of the depth hoar were frequently hollow, or had internal, parallel arrays of facets or steps, which resulted from the refreezing of successive molecular layers of ascending water vapor (Figure 12). Unlike precipitated snow, depth hoar crystals generally were not hexagonally symmetrical. Furthermore, the depth hoar was not significantly sintered or bonded to adjacent crystals.

In spring, the snowpack in Colorado was subjected to day/night fluctuations in temperatures that produced changes referred to as melt-freeze metamorphism.

**Figure 10.** Rounded and well-bonded snow crystals that result from long exposures to low temperature gradients. Sample was collected from a snowpit 19 cm below the surface near Loveland Pass, Colorado, elevation 3600 m ASL.

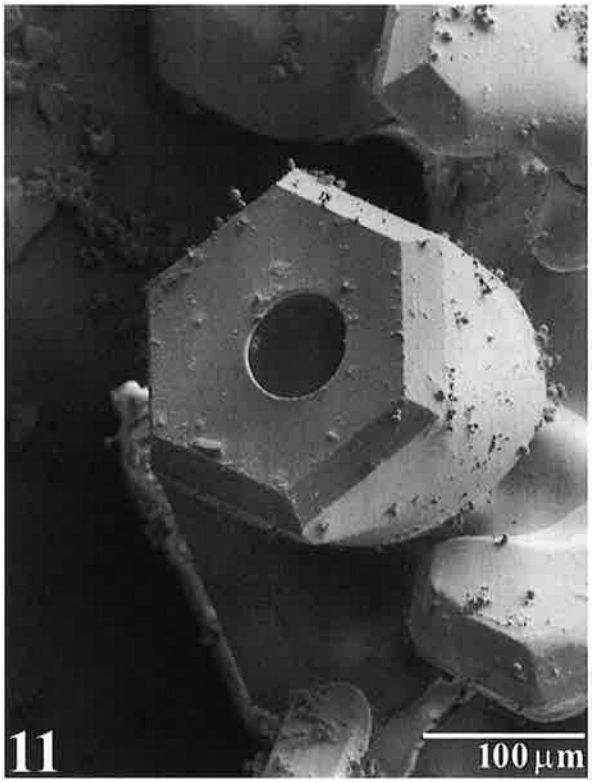


**Figure 11.** Large crystal that exhibits rounding and faceting and is believed to result from both low (rounding) and high (faceting) temperature gradients in the snowpack. Sample obtained near Jones Pass (Henderson Mine), Colorado, elevation 3150 m ASL, air temperature -6°C. Collected at 84 cm below the surface.

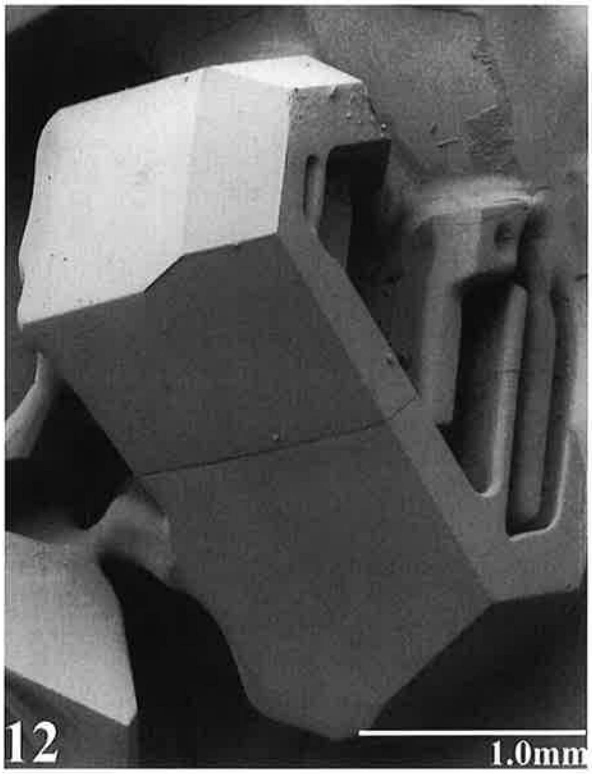


**Figure 12.** Large depth hoar crystal. Sample was collected 90–100 cm below the surface of 1m snowpit at Ester Dome, near Fairbanks, Alaska, elevation 700 m ASL, air temperature +4°C.

**Figures 13–16.** Samples were collected near Loveland Pass, Colorado, elevation 3600 m ASL, air temperature +18°C, shipped to the laboratory and stored in *l*$N_2$ prior to imaging.

**Figure 13.** Melting surface layer of a snowpack. A film of water appears to bond the rounded snow grains into a cluster.

During the day when the temperature was above freezing, this process resulted in the formation of spherical snow grains that were covered with a film of surface water, and consequently, were only weakly bonded to one another. However, at night when the temperature was below freezing, the water film froze and the grains became well-bonded to one another. Our sampling procedure, which refroze the surface water, produced images that simulated those found at night (Figure 13). Lightly etching the surface of this type of snow sample revealed different morphological patterns resulting from dissimilarities between the water-ice of the crystals and that formed by the film of surface water that was frozen during the sampling procedure.

### Red Snow

In Colorado, the surface of the snowpack that was undergoing melt-freeze metamorphism exhibited a reddish coloration. Fracturing surface samples from this snowpack revealed numerous cross sections of circular structures measuring about 5 to 25 Ëm in diameter (Figure 14). These structures, which occurred just beneath the uppermost film of water, could also be exposed by etching a few microns of water-ice from the surface of the sample. The structures appeared to resemble cells, had a peripheral layer consisting of densely packed granules but showed no significant evidence of the formation of internal ice crystals (Figure 15).

To help further identify these structures, the samples that were observed with LTSEM were removed and processed, using freeze-substitution, for TEM observations. Cross-sections of these structures exhibited a large central chloroplast with numerous starch granules that tended to be localized around the periphery. The parietal layer of cytoplasm contained a nucleus, mitochondria, dictyosomes, ribosomes, and several types of vesicles (Figure 16). Because the presence of these cells was not expected at the time the snow was collected, no additional samples were taken in an attempt to isolate, culture, or positively identify this organism.

## 4. DISCUSSION

Results from this study indicate that snow samples can be successfully collected at remote locations, stored and shipped to a laboratory for observation with LTSEM. The structural details of these snow crystals were similar to those from newly precipitated samples that were previously obtained at the laboratory site and directly observed by this technique (Wergin and Erbe, 1974a; 1994b; 1994c; Wergin et al., 1995a; 1995b). These observations suggest that neither the storage conditions nor the shipping procedures affect the general structure of precipitating snow crystals. Because the metamorphosed snow samples observed in this study were handled in the same manner, we feel that their structural features were not altered.

Observing and recording images of snow crystals with LTSEM has numerous advantages over similar studies using light microscopy (Bentley, 1931; Nakaya, 1954) or hand lenses and photomacrography (LaChapelle, 1969; Armstrong, 1992; McClung and Schaerer, 1993). In light microscopic studies, samples must generally be observed and photographed at field sites in subzero temperatures or in specially designed cold laboratories. The classical photographs of Bentley (1931) were taken in an outdoor shelter, whereas Nakaya (1954) constructed a laboratory that was maintained at -30 to -45°C for this purpose. Even at these temperatures, humidity, body heat, sample illumination, and variable pressure gradients can affect melting, recrystallization, or sublimation of the sample. Alternatively with LTSEM, cryofixation, storage, transportation, and observation of samples are accomplished at $lN_2$ temperatures. Therefore: 1) melting was not a problem; 2) recrystallization does not occur (Robards and Sleytr, 1985); and 3) sublimation in the SEM, which was calculated to be less than $10^{-13}$ cm/sec at -150°C (Robards and Sleytr, 1985), would not be detectible. Although the cost of equipment required for LTSEM is greater than that needed for light microscopy, the former technique does not require construction of a cold laboratory on site, and the basic procedures are similar to those routinely used for observations of frozen, hydrated biological specimens (Wergin et al., 1996).

Information obtained from LTSEM observations was less confusing and easily surpassed that achieved through examination with the light microscope or a hand lens. The LTSEM provided surface information that was not confused by any internal structure of the crystals. Internal structure could be observed if the crystals were cryofractured. Alternatively, the illustrations obtained with light microscopy (Bentley, 1931; Nakaya, 1953), which used transmitted or oblique illumination, respectively, provided images that were composed of surface as well as internal structures. As a result, surface patterns could not be readily distinguished from internal features. For example, Bentley described "bubbles" in the dendritic crystals, which we believe corresponded to "negative crystals" (Hobbs, 1974) that were commonly present on the surface of the dendrites that we observed.

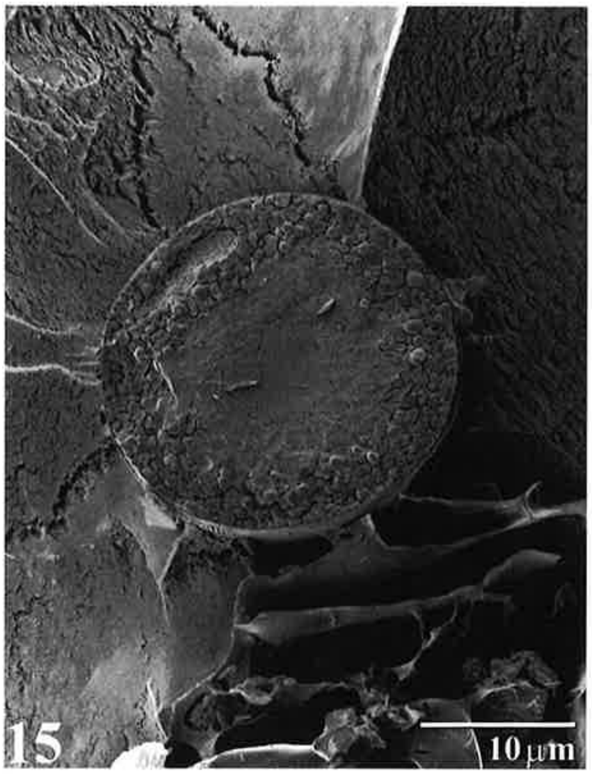
The LTSEM also has a depth of field several orders of magnitude greater than that of the light microscope; furthermore, this instrument allowed us to record and view true, three-dimensional images of the specimens. This greater depth of field allowed clear, in-focus images of large crystals such as depth hoar and graupel, which had considerable surface topography. Furthermore, three-dimensional images provide the potential to obtain quantitative data (by stereometry), such as crystal size and volume. Neither of these features have been reported in light microscopic investigations. Finally, many of the images that are illustrated in the present study were recorded at magnifications similar to those that have been photographed with a light microscope; however, the resolution of the LTSEM even at magnifications of only a few hundred times was much greater than that which has been published for light micrographs. Consequently, rime droplets and "skeletal" structures, which could only be surmised or illustrated by line drawings in the light microscopic investigations, can now be clearly illustrated with images obtained in the LTSEM.

**Figure 14.** Fractured surface of melting snow. Sample was also etched to sublime the surface water-ice. This procedure reveals the presence of small spherical bodies believed to be a green alga, possibly *Chlamydomonas nivalis.*

## Comparisons of Crystal Types

Although storage and transport did not appear to adversely affect the structure of the snow crystals, the types of newly precipitated crystals that were observed in the samples from Maryland and West Virginia did differ; hexagonal plates and needles were present in the Maryland samples, and graupel and dendritic forms were found in the West Virginia snow. These differences are not believed to be related to sampling procedures, differences in air temperatures, or storage conditions, but are probably associated with variations in the cloud temperatures that prevailed during crystal formation. This observation would be consistent with those of other investigators who concluded that the shapes of newly formed snow crystals are predominantly influenced by the temperatures at which they were formed (Nakaya, 1954; Hobbs, 1974; Mason, 1992).

The samples from Colorado and Alaska were chosen to illustrate the metamorphism that occurs in the snowpack. The observations of depth hoar crystals, which develop under high temperature gradients, were consistent with the observations that had been previ-



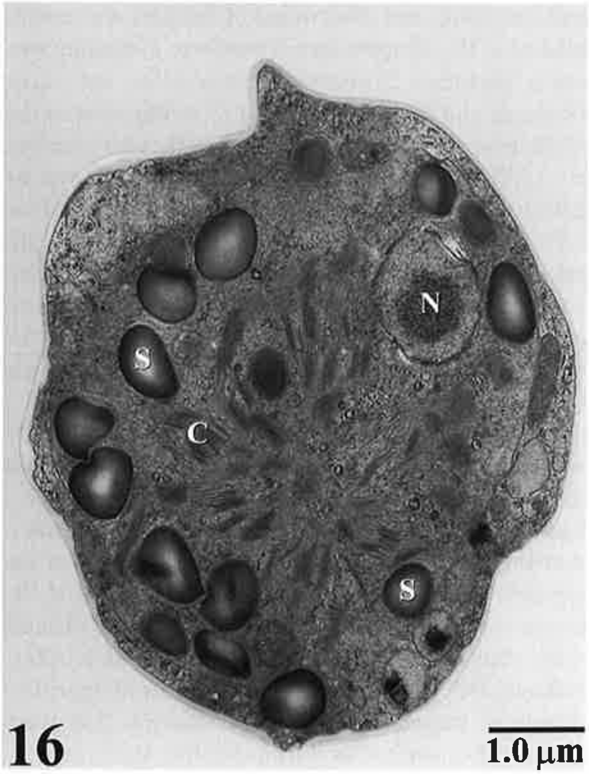


**Figure 15.** Fractured and etched surface of melting snow illustrating a single cell believed to be a green alga causing the "red snow" phenomenon.

**Figure 16.** Transmission electron micrograph of an algal cell from one of the samples of red snow that had been observed in the LTSEM. The cell exhibits fine-structural features characteristic of a green alga. These include the large central chloroplast (C) with numerous starch (S) granules and a peripheral layer of cytoplasm containing a nucleus (N), mitochondria, ribosomes, and vesicles.

ously made by light microscopy (Armstrong, 1992): namely, the depth hoar consists of crystals that attain sizes of several mm, are highly faceted, hollow, and show little or no sintering or bonding to adjacent snow crystals. Alternatively, low temperature gradients lead to snow crystals that are rounded or sinuous and highly sintered; the snowpack has smaller air spaces than those that developed under high temperature gradients (Armstrong, 1992). These characteristics were well-preserved in the samples that were obtained from the snowpack in Colorado (Figure 9).

Finally, melt-freeze metamorphism, which normally occurs in spring, results in spherical snow crystals that are bonded in clusters; the strength of the bonds is affected by the amount and status of water that is present (McClung and Schaerer, 1993). Such spherical snow crystals were evident in the surface formations that were collected in spring from Colorado. Although these samples where obtained under melt conditions during the day, plunge freezing them in liquid nitrogen froze the film of surface water that was present during the day melt. Etching these samples helped to distinguish the two types of water-ice that were present.

## Red Snow

During spring in alpine regions, the surface of the snowpack frequently has a colored appearance consisting of light hues of green, salmon, orange, or red. The coloration is generally associated with the growth of a specific species of algae, which, along with many other organisms, can be found near the surface of the melting snowpack. Hoham (1992) reported that in western North America the appearance of red snow was caused by the motile unicellular green alga *Chlamydomonas nivalis*. The red coloration resulted from the high accumulation of carotenoids, especially astaxanthin, that occurs in certain alpine environments. The carotenoids probably function as photoprotectants for chlorophyll under the high irradiation levels that occur at high altitudes (Czygan, 1970). Our summer collection site was similar to that surveyed by Hoham. When the cells that we encountered in the red snow samples were prepared and observed in the TEM, they exhibited fine structural features that were very similar to those recently described for another *Chlamydomonas* species, namely *Chlamydomonas reinhardtii* (see Figure 3(a), Pérez-Vicente et al., 1995). Although we did not culture these cells for taxonomic identification, the previous survey by Hoham and the fine structural features observed in our TEM observations suggest that the cells found in our sample probably represent a species of *Chlamydomonas*. The LTSEM technique may allow *in situ* studies of these and other microorganisms that are commonly found in the spring snowpack.

### Importance of Snow Crystal Structure

Snow, which may cover up to 53% of the land surface in the Northern Hemisphere (Foster and Rango, 1982) and up to 44% of the world's land areas at any one time, supplies at least one-third of the water that is used for irrigation and the growth of crops (Gray and Male, 1981). For this reason, calculating the quantity of water that is present in the winter snowpack is an extremely important forecast activity that attempts to predict the amount of water that ultimately will reach reservoirs and estimates how much water will be available for agricultural purposes during the following growing season. In 1980, Castruccio et al. (1980) estimated that improving predictions by 1% would result in a $38 million irrigation and hydropower benefit for states in the western U.S.A. Today this figure has probably doubled as a result of inflation. Remote sensing approaches using microwave data have been successfully tested in certain situations to calculate areal water equivalent of the snowpack prior to melting (Rango et al., 1989; Goodison et al., 1990). Unfortunately, these estimates can be easily confounded by the sizes and shapes of the snow crystals or grains that constitute the snowpack. Our results indicate that low temperature SEM can be used to illustrate the sizes and shapes of snow crystals and to follow their subsequent metamorphisms influenced by temperature, relative humidity, vapor pressure, and time. Current microwave radiative transfer models assume that the snow crystal is spherical. Our information concerning the shapes of snow crystals can now be used in developing new radiative transfer models to more accurately determine microwave scattering in the snowpack and thereby assess the water content of the snow that is present.

In conclusion, LTSEM provides a new technique for observing various types of newly precipitated and metamorphosed snow crystals that can be collected, stored, and shipped from remote sites. The technique has a resolution and depth of field that cannot be achieved with the light microscope and also permits quantification using stereological approaches. In addition, application of this technique can be used to observe the process of sublimation of snow crystals (Wergin et al., unpublished), to gain information about icicles, ice fabric, and frost (Wergin et al., 1996), and offers the potential for X-ray analysis to acquire data about the elemental composition of condensing nuclei and particulate pollutants that may become incorporated into snow and ice.

## 5. ACKNOWLEDGMENTS

The authors thank Christopher Pooley for converting the SEM negatives to the digital images that were used to illustrate this study. We thank Richard Armstrong and Rod Newcomb for assistance in selecting appropriate snowpits in Colorado, and Anne Nolin and Beth Boyer for assisting in data collection at Loveland Pass. We were also assisted by Dorothy Hall, Jim Foster, and Carl Benson in the snowpits near Fairbanks, Alaska. This investigation was partially funded by the NASA Goddard Space Flight Center.

## REFERENCES

**Armstrong, R. L.** (1992) The mountain snowpack: pp. 47–83, in: *The Avalanche Book*, ed. by B. R. Armstrong and K. Williams, Colorado Geological Survey, Denver.

**Bentley, W. A. and Humphreys, W. J.** (1931) *Snow Crystals*, McGraw-Hill Book Co., Inc., New York.

**Blanchard, D. C.** (1970) Wilson Bentley, the snowflake man, *Weatherwise*, 23, 260–269.

**Brownscombe, J. L. and Hallet, J.** (1967) Experimental and field studies of precipitation particles formed by the freezing of supercooled water, *Quarterly Journal of the Royal Meteorological Society*, 93, 455–473.

**Castruccio, P. A., Loats, H. L., Lloyd, D., and Newman, P. A. B.** (1980) Cost/benefit analysis for the operational applications of satellite snowcover observations (OASSO): pp. 239–254, in: *NASA Conference Publication 2116*, ed. by A. Rango and R. Peterson, Scientific Technical Information Office, Springfield, Virginia.

**Czygan, F. C.** (1970) Blutregen und Blutschnee: Stickstoff-mangel-Zellen von *Haematoccus pluvialis* und *Chlamydomonas nivalis, Archive Mikrobiologie*, 74, 69–76.

**Dobrowolski, A.** (1903) La neige et le givre. Expédition Antarctique Belge: pp. 1–78, in: *Résultats du voyage du S.Y. Belgica en 1897-1898-1899*, ed. by J. E. Buschmann, Antwerp, Belgium.

**Foster, J. L. and Rango, A.** (1982) Snow cover conditions in the northern hemisphere during the winter of 1981, *Journal of Climatology*, 20, 171–183.

**Gray, D. M. and Male, D. H.** (1981) *Handbook of Snow: Principles, Processes, Management and Use*, Pergamon Press, Toronto, Canada.

**Goodison, B., Walker, A. E., and Thirkettle, F.** (1990) Determination of snow water equivalent on the Canadian prairies using near real-time passive microwave data: pp. 297–316 in *Proceedings Workshop on Application of Remote Sensing in Hydrology*, National Hydrology Research Institute, Saskatoon, Canada.

**Hellman, G.** (1893) *Schneekrystalle*, J. Muckenberger, Berlin.

**Hobbs, P. V.** (1974) *Ice physics*, Clarendon Press, Oxford, England.

**Hoham, R. W.** (1992) Environmental influences on snow algal microbes: pp. 78–83, in *Proceedings of 16th Annual Western Snow Conference*, ed. by B. Shafer, Jackson Hole, Wyoming.

**LaChapelle, E. R.** (1969) *Field Guide to Snow Crystals*, University of Washington Press, Seattle.

**Mason, B. J.** (1992) Snow crystals, natural and man-made, *Contemporary Physics*, 33, 227–243.

**McClung, D. and Schaerer, P.** (1993) *The Avalanche Handbook*, The Mountaineers, Seattle.

**Nakaya, U.** (1954) *Snow Crystals*, Harvard University Press, Cambridge, Mass.

**Nordenskiold, G.** (1893) The inner structure of snow crystals, *Nature Land*, 48, 592–594.

**Pérez-Vicente, R., Burón, M. I., González-Reyes, J. A., Cárdenas, J., and Pineda, M.** (1995) Xanthine accumulation and vacuolization in *Chlamydomonas reinhardtii* cells, *Protoplasma*, 186, 93–98.

**Rango, A., Martinec, J., Chang, A. T. C., and Foster, J. L.** (1989) Average areal water equivalent of snow in a mountain basin using microwave and visible satellite data, *I.E.E.E. Transactions Geoscience and Remote Sensing*, 27, 740–745.

**Robards, A. W. and Sleytr, U. B.** (1985) *Low Temperature Methods in Biological Electron Microscopy*, Elsevier, Oxford, England.

**Stoyanova, V., Genadiyev, N., and Nenow, D.** (1987) An application of the replica method for SEM study of the ice crystal instability, *Journal of Physics* (Paris), 48, 375–381.

**Takahashi, T. and Fukuta, N.** (1987) *Journal of Physics* (Paris), 48, 405–411.

**Wergin, W. P. and Erbe, E. F.** (1994a) Can you image a snowflake with an SEM? Certainly! *Proceedings of the Royal Microscopy Society*, 29, 138–140.

**Wergin, W. P. and Erbe, E. F.** (1994b) Snow crystals: Capturing snowflakes for observation with the low temperature scanning electron microscope, *Scanning*, 16, IV88–IV89.

**Wergin, W. P. and Erbe, E. F.** (1994c) Use of low temperature scanning electron microscopy to examine snow crystals: 3B: pp. 993–994, in: *Proceedings of 14th International Congress of Electron Microscopy* (ICEM 13), ed. by B. Jouffrey and C. Colliex, Les Editions de Physique Les Ulis, Paris, France.

**Wergin, W. P., Rango, A., and Erbe, E. F.** (1995a) Observations of snow crystals using low-temperature scanning electron microscopy, *Scanning*, 17, 41–49.

**Wergin, W. P., Rango, A., and Erbe, E. F.** (1995b) Three dimensional characterization of snow crystals using low temperature scanning electron microscopy, *Scanning*, 17, V29–V30.

**Wergin, W. P., Rango, A., and Erbe, E. F.** (1996) Use of low temperature scanning electron microscopy to observe icicles, ice fabric, rime and frost, in: *Proceedings of the Microscopy Society of America and Analysis '96*, ed. by G. W. Bailey et al., Jones and Begell Publishing, New York, in press.

**Wergin, W. P., Yaklich, R. W., and Erbe, E. F.** (1996) Advantages and applications of low temperature scanning electron microscopy, in: *Focus on Modern Microscopy*, World Scientific Publishing Co., Ltd., New Jersey, in press.

**Wolff, E. W. and Reid, A. P.** (1994) Capture and scanning electron microscopy of individual snow crystals, *Journal of Glaciology*, 40, 195–197.