

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 \times .

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 \times , 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

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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).

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 macromolecular 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 LN₂ 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 LN₂ to freeze, store, and ship samples may be too harsh or extreme and that imaging with LTSEM may alter structure of the sample. However, LN₂, 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 LN₂. Because LN₂ 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 LN₂.

To further document that coating and imaging samples with LTSEM do not affect the structure, individual 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 CO₂ 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).

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.