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Citation	Review of Scientific Instruments, 80(11), 115102 https://doi.org/10.1063/1.3222739
Issue Date	2009-11
Doc URL	https://hdl.handle.net/2115/42548
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Type	journal article
File Information	RSI80-11_115102.pdf

Apparatus for single ice crystal growth from the melt

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A crystal growth apparatus was designed and built to study the effect of growth modifiers, antifreeze proteins and antifreeze glycoproteins (AFGPs), on ice crystal growth kinetics and morphology. We used a capillary growth technique to obtain a single ice crystal with well-defined crystallographic orientation grown in AFGP solution. The basal plane was readily observed by rotation of the capillary. The main growth chamber is approximately a 0.8 ml cylindrical volume. A triple window arrangement was used to minimize temperature gradients and allow for up to 10 mm working distance objective lens. Temperature could be established to within ±10 mK in as little as 3.5 min and controlled to within ±2 mK after 15 min for at least 10 h. The small volume growth chamber and fast equilibration times were necessary for parabolic flight microgravity experiments. The apparatus was designed for use with inverted and side mount configurations. © *2009 American Institute of Physics.* [doi:10.1063/1.3222739]

I. INTRODUCTION

Antifreeze proteins (AFPs) and antifreeze glycoproteins (AFGPs) help fish, insects, plants, and bacteria survive exposure to subfreezing environments. These proteins modify the growth habits, inhibit the recrystallization and the growth of ice and have drawn a considerable interest due to their applicability in the frozen food industry,[1,2] protection of plants that have been transgenetically mutated to express these proteins,[3] as a cryosurgery adjuvant,[4] in rat islet preservation, and even the possibility of human organ preservation.[5,6] Although it is clear that the proteins carry out their functions via some surface interaction, there is still much debate as to the exact details of the mechanism.[7] Additionally, to date no thorough quantitative description of the ice crystal growth kinetics changes due to the protein interactions has been reported and such studies could lead to a better understanding of the anisotropic, binding site, or plane specific nature of the interaction.

To this end we have developed a melt ice crystal growth apparatus to study the growth morphology changes and ice crystal growth kinetics with the AFPs as well as the protein kinetics at the ice interface.[7] The apparatus was developed for use with laser scanning confocal fluorescence microscopy, phase contrast microscopy, and interference microscopy in an inverted configuration as well as a side-mount configuration. Altogether these techniques can provide simultaneous a-axis and c-axis growth rate measurements and even nanoscale surface evolution unavailable with conventional bright field microscopies previously used to image ice crystal growth. In addition, this growth cell was designed to study the effects of microgravity on ice crystal growth from AFGP solutions during parabolic flights. These experiments require a relatively compact and semiportable system with excellent repeatability (near 100%). The microgravity period lasts 20 s at 6 min intervals for 1 h.

Our apparatus is similar in concept to that of Tirimizi

et al.[8] and Furukawa et al.,[9] among others. In these systems, single crystals were readily grown from a capillary into solution and we have adopted this capillary growth technique. With pure water, the c-axis will grow perpendicular to the capillary axis nearly every time and hence little manipulation of the crystal itself is necessary for imaging. The temperature in these systems was readily controlled with circulating baths and could achieve temperature stabilities within 15 mK.[8] However, the time required to reach a target temperature and stabilize is up to 1 h or more and much too slow for our purposes.[9] Our system uses thermoelectric elements for temperature control with sample volumes less than 1 ml. In addition, we have minimized the thermal mass and can reach the target temperature within as little as 3.5 min with temperature variation better than 8 mK across the solution. Additionally, the system used by Furukawa et al.[9] required telescope optics since the sample to outer window distance was 85 mm. The objective lenses that we use have working distances of 16 and 10 mm; therefore, we designed our growth apparatus to have a sample to outer window distance less than 10 mm.

A similar apparatus has been used for pure water ice crystal growth during microgravity experiments in a drop shaft[10] as well as a smaller version in the International Space Station.[11] In principal, there are two differences in our design: (1) the capillary is now mounted on a rotating capillary holder to manipulate the crystal orientation and (2) cold liquid spray is used for nucleation rather than a separate nucleation chamber. In this paper, we will give a detailed description of our apparatus.

II. GROWTH CHAMBER

The sample chambers of previous systems were between 10 and 100 ml. Such large volumes are inadequate for two reasons. (1) The large volumes can require up to 1 h or more for thermal equilibration. In-flight, the target time between
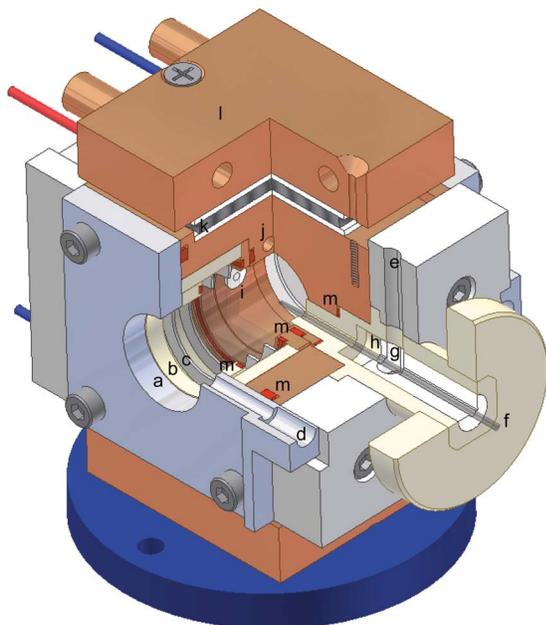
FIG. 1. (Color online) Drawing of the growth apparatus for the side mount interferometer configuration. (a) Teflon® cover holding the window arrangement. (b) Wedge piece used to adjust the window substrates reflection away from the image plane. (c) Outer flat glass substrate. (d) N_2 feed port used to keep outer window surface dry. (e) Port used to feed cold liquid for ice nucleation. (f) Glass capillary. (g) Cold liquid is directed here for nucleation. (h) Void filled with aerogel to help insulate from overcooling during nucleation. (i) Solution exchange and temperature monitor thermistor port for configuration 1 and temperature control thermistor port for configuration 2. (j) Control temperature thermistor port for configuration 1. (k) Thermoelectric cooling elements. (l) Heat exchanger element connected to circulating bath. (m) O-rings shown are used to contain the solution and to keep the air between the glass substrates dry.

microgravity experiments was 6 min. (2) One of our objectives is to investigate the AFGP mechanism and thus 10–100 ml of solution would require far too much sample. This could run too costly per experiment or even impossible for some rare types of AFPs. In our design, the sample chamber volume is less than 1 ml. This allows for ample space to adequately study the free melt growth of ice to supercoolings as low as 1 K.

The principal component is a 40 mm cube of oxygen free copper, Fig. 1. We removed the maximum amount of copper minimizing the thermal mass in order to reach the target temperature and stabilize as quickly as possible. The growth chamber is a cylinder 10 mm in height and diameter. This was strategically placed so that the center of the growth chamber, at the capillary location, is 10 mm from the outer window [Fig. 1(c)] of the objective side. The optical path length is longer due to the windows and solution, effectively increasing the actual working distance of the objective by 3 mm and can accommodate objective lenses with air working distances of 7 mm. There are two ports aligned normal to the chamber radius that serve multiple purposes. Both are necessary for solution exchange. During the experiment the capillary holding device is placed in one port while the other [Fig. 1(i)] is used for pressure relief caused by the expanding volume during growth of the solid. A thermistor used to measure the solution temperature during the experiment is inserted through the pressure relief port. All of the copper is

coated with Teflon®.

III. TRIPLE WINDOW ARRANGEMENT

A triple window setup was chosen to best insulate the optical port and arranged as deep into the copper as possible to achieve the best isothermal conditions as quickly as possible. For the phase contrast microscope, we used o-ring spacers (flat Teflon® spacers were used for the interferometer arrangement) with the window surface arranged normal to the optic axis on both the objective and illumination sides. It was also necessary to use antireflective (AR) coated windows since any slight misalignments of the windows caused reflections to miss the phase plate deteriorating the phase contrast image quality. However, AR coating was not sufficient for the Michelson interferometer arrangement. The reflectivity of ice in water is much lower than the AR coating and is not visible when the window interference fringes are present in the image. Wedge spacers were introduced on the ends of the triple window arrangement on both sides of the growth chamber to divert the reflections away from the image. Alternatively, the entire growth apparatus can be tilted to achieve the same result. Additionally, it is also helpful to have rotational capabilities about the azimuth since the ice crystal c-axis orientation can result a few degrees from perpendicular to the capillary making interferometer alignment difficult.

This window arrangement was held in place by a Teflon® cover that also served to eliminate condensation on the main copper chamber [Fig. 1(a)]. To avoid condensation on the windows, it was also necessary to keep the air at the window surfaces dry. Thus, the window assembly took place in a nitrogen purged glove box or glove bag. During experiments N_2 gas was fed to the outer surface of the windows through ports [Fig. 1(d)] in the Teflon® covers or N_2 purged plastic bag enclosures were used. However, during the dry winter months (in Sapporo), the dew point usually drops to temperatures well below the experimental temperatures making these precautions unnecessary.

IV. CAPILLARY

The capillary growth method has been used to grow single crystals in solution by several groups.[8,9,12,13] It becomes especially important to have single crystalline samples when studying the growth kinetics. With batch growth techniques, the supercooling at the crystal interface can vary considerably. In the case of ice, the large amounts of heat generated will change the growth conditions, making it nearly impossible to know the actual solid/solution interface temperature. A simple reading of the control temperature does not suffice here. With single crystal growth measurements, the temperature is closer to the solution temperature, and the profile is less ambiguous and can more accurately be determined. Here, micropipette capillaries were shaped and then mounted onto a capillary holder.

A. Capillary pulling

To some extent the inner diameter of the capillary will determine the initial thickness of the crystal and the thick-
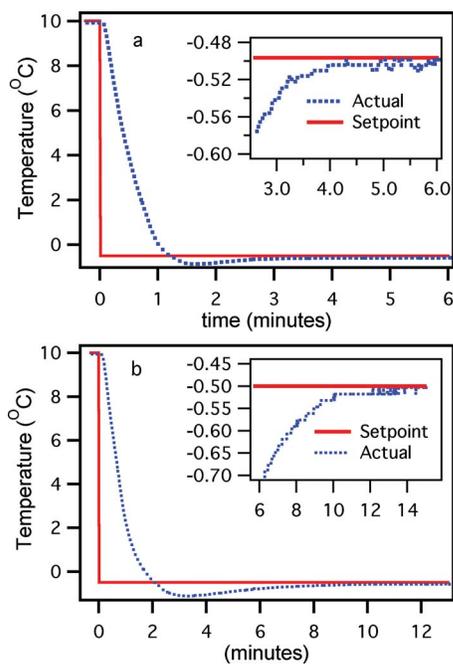
FIG. 2. (Color online) Temperature profiles with the control thermistor (a) inside the copper and (b) inside the solution. Inset is a fine scale view when the temperature approaches the set point temperature.

ness to within the time frames studied here. Additionally, a tapered capillary will help reduce growth back along the capillary toward the chamber walls. When the ice reaches the chamber walls, the large amounts of growth will cause the same effect as secondary nucleation (see Sec. IV B), heating the solution and ending the experiment. To reduce these effects, we shaped our capillaries by pulling, grinding, and flame annealing the end. Other much more eloquent techniques have been developed and are commercially available to pull micropipette glass capillaries. Here, we will briefly describe a simple and inexpensive technique that was more than adequate for our purposes.

The micropipette glass capillary was held on both ends with silicone tubing under slight tension. A portable bottled torch was used to heat the capillary and pulled by the tension in the tubing to diameters of a few tens of microns. However, the smallest diameter was far too fragile for our purposes. The most symmetric capillaries were achieved by not allowing the capillary to break during this step. At this step the capillary inner diameter was typically reduced from 1 mm to $15-20$ μm. The capillary was then broken with tweezers and the ends were ground with a 1600 grit surface to remove the sharp edges. During this step, a small flow of N_2 was injected on the other end with silicone tubing line in order to avoid debris from getting inside the capillary. This rough surface was then flame annealed to obtain a smooth surface and during this step we could further reduce the inner diameter at the end to less than 5 μm.

B. Capillary holder

A capillary holder made from PEEK® (polyetheretherketone) was designed for durability and easy manual rotation, Fig. 1. The capillary was held in place with epoxy and ar-

ranged so that a 1 mm crystal attached at the end would be centered in the chamber. An o-ring was used to seal the copper/PEEK® contact. Similar to the window arrangement, the capillary holder was held in place by a Teflon® cover, as shown in Fig. 1. Ice was nucleated by inverting an air duster to eject the liquid and directing it to the capillary through a port in the cover [Fig. 1(e)] and capillary holder [Fig. 1(g)]. With this nucleation process, it is possible to overcool the main copper chamber through the capillary holder assembly and cause secondary nucleation on the chamber walls. The heat generated by the ice growing on the chamber walls is sufficient to raise the solution temperature to near the melting point, changing the experimental conditions in the solution. Although this generally could be avoided by limiting the amount of liquid injected to the holder, the capillary was further insulated from the chamber by a small void that was filled with aerogel. This worked well enough that during flight it was not necessary to control the amount of liquid used to nucleate the ice.

C. Ice nucleation

At the point of nucleation, many ice crystals are formed. In short, a competition takes place between the multiple grains at the growing interface advancing toward the solution. The less obstructed grain with the fast growth axis oriented parallel to the capillary axis will exit the capillary into the solution. Off-axis grains get reflected from the capillary walls slowing down their propagation along the capillary and eventually get blocked by the faster grains. The distance from the nucleation point to the capillary exit was 25 mm, sufficient to give a crystal with the c-axis oriented perpendicular to the capillary length. Ice crystals typically exited the capillary into the solution quite reproducibly approximately $1-3$ min after nucleation depending on the solution supercooling. Essentially all the crystals were oriented with the a-axis parallel to the capillary, especially at the lower protein concentrations (<1 mg/ml). AFPs modify the crystal growth kinetics anisotropically and at higher concentrations growth can be fastest along the c-axis.

V. TEMPERATURE CONTROL

Temperature control was carried out with thermoelectric elements [Fig. 1(k)] powered by a commercially available temperature controller, (Melcor, Trenton, NJ). Heat was drawn from the cooling elements with copper heat exchangers [Fig. 1(l)] connected to a circulating bath. We chose the circulating bath temperature to be slightly above the dew point to avoid water condensation. Temperature was measured inside the solution using a thermistor with a commercially available temperature monitor (Cryocon, Model 14, Rancho Santa Fe, CA). For accurate temperature measurements, thermistors were calibrated using a water triple point cell with conditions where all three phases, solid, liquid, and vapor, were present, i.e., at the triple point 0.01 °C.

The main difference from the previous designs is that here the fluid bath is used for heat exchange and not temperature control. The fluid bath is usually liters of liquid and all of this must stabilize in temperature as well as the glass
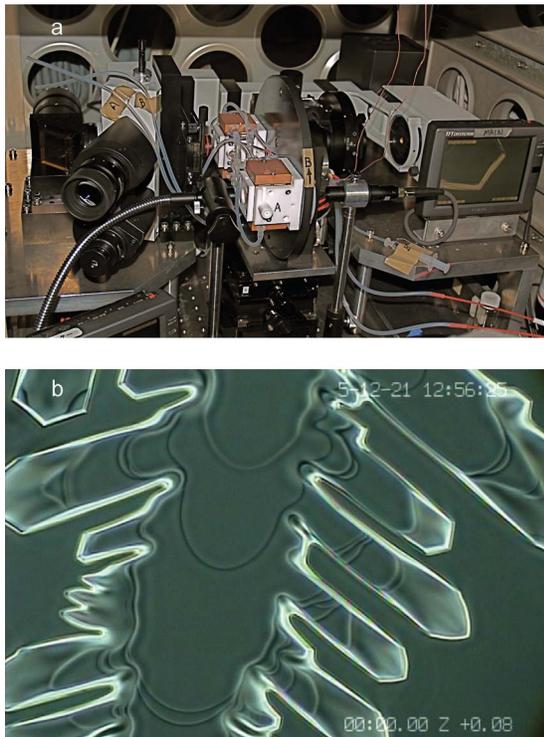
FIG. 3. (Color online) (a) Side mounted phase contrast microscope with the rotating double growth apparatus configuration in the experimental rack inside the jet airplane. Growth apparatus pictured is the third-generation design; fourth generation is shown in Fig. 1. (b) Dendritic ice crystal growing in AFGP solution at −0.7 °C during microgravity ($z=+0.08$ g). The c-axis is normal to the plane. Note the highly faceted structure and steps visible on the basal plane.

growth chamber material and finally the solution, between 10 and 100 ml. In our configuration, the only thermal mass is the copper growth chamber and about 1 ml of solution that is controlled electronically, while the fluid bath temperature is fixed since it is only used for heat exchange.

VI. RESULTS

To determine the performance of our apparatus, we measured the time required for thermal equilibration as well as the temperature stability over extended periods. Two approaches were taken to control the temperature. First, the temperature was controlled with the control thermistor inside the main copper block 1.5 mm from the solution, Fig. 1(j). The initial temperature was 10 °C, since this is a typical temperature that we would use to melt ice from previous experiments during a microgravity flight for example. Figure 2(a) shows the temperature during the cooling process. The temperature comes to within 15 mK of the set point temperature in 3.5 min, and stabilizes after 8 min with fluctuations less than ±3 mK for over 12 h. However, the actual solution temperature measured in the solution was seen to fluctuate between ±8 mK over 1 h and within ±15 mK over a 12 h period. The second approach was to control the temperature inside the solution itself, by placing the control thermistor within the solution. From Fig. 2(b), we can see that the main

drawback to this scenario is the time required to reach the target temperature. It took 10 min to come within 15 mK of the target temperature, but after 15 min the temperature was ±2 mK for over 4 h. In both cases, the temperature variations across the solution were comparable to the stability measured at the center of the growth chamber.

Both temperature control approaches achieve stable enough conditions. The first reaches the target temperature quickly while the second is more stable. Therefore, for experiments of supercoolings less than 0.1 K, control within the solution is more appropriate. However, the equilibration times required in the second scenario were longer than desired for our microgravity experiments and the first approach was used for the in-flight experiments. Note that the goal is to run an experiment during the microgravity period (20 s), melt back the ice, and prepare the growth apparatus for the next experiment all within 6 min. Finally, we arranged a double growth apparatus configuration for the phase contrast microscope to maximize readiness between experiments. Two growth apparatus were mounted on a rotating stage, Fig. 3(a), to give ample preparation time between experiments for each of the growth apparatus. For the interferometer arrangements, we were unable to use a similar double growth apparatus arrangement due to the sensitivity of the interferometer alignment required to obtain interference fringes from the ice surface.

Figure 3(b) shows an ice crystal grown in AFGP solution during the microgravity period.

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