# DEVELOPMENT OF THREE-DIMENSIONAL MEASUREMENT METHOD FOR ICE CRYSTALS IN FROZEN LIQUID FOODS

○ SHIGEAKI UENO, YASUYUKI SAGARA, KEN-ICHI KUDOH* and TOSHIRO HIGUCHI*

Department of Global Agricultural Sciences, The University of Tokyo,
1-1-1 Yayoi, Bunkyo-ku, Tokyo 1138657, Japan
*Department of Precision Machinery Engineering, The University of Tokyo,
7-3-1 Hongo, Bunkyo-ku, Tokyo1138658, Japan
Corresponding author: SHIGEAKI UENO, The University of Tokyo,
Tel.: +81-3-5841-7536,Fax: +81-3-5841-5335, E-mail address: *aa27126@mail.ecc.u-tokyo.ac.jp*

## ABSTRACT

A Micro-Slicer Image Processing System (MSIPS) has been applied to observe the ice crystal structures formed in frozen dilute solutions. Several characteristic parameters were also proposed to investigate the three-dimensional (3-D) morphology and distribution of ice crystals, based on their reconstructed images obtained by multi-slicing a frozen sample with the thickness of 5μm. The values of characteristic parameters were determined for the sample images with the dimension of 530 x 700 x 1000μm. The 3-D morphology of ice crystals was found to be a bundle of continuous or dendrite columns at any freezing condition. The equivalent diameter of ice crystals were in the range of 73μm to 169μm. At a temperature of a copper cooling plate of -40℃, the volumes of ice crystals were in the range of 4.6 x $10^4$μ$m^3$ to 3.3 x $10^7$μ$m^3$, and 36 ice columns were counted in the 3-D cubic image.

*Key words:* Freezing; Ice crystal; Dilute solution; Three-dimensional; Micro-Slicer Image Processing System; Internal structure microscopy

## INTRODUCTION

Direct observation methods of ice crystals include cryo-scanning electron microscopy, cold microscopy and confocal laser scanning microscopy. Indirect methods such as freeze substitution, freeze fixation and freeze-drying technique are based on the hypothesis that the void having original ice crystal morphology is maintained after substitution and sublimation. However, these techniques still have disadvantages, because the micro structures would have changed in the vicinity of sublimination process. Although it is valuable to apply all these methods for observing two-dimensional or cross-sectional characteristics such as ice crystal size and distribution, the 3-D morphology of ice crystals is still unable to be analyzed quantitatively.

A Micro-Slicer Image Processing System (MSIPS) was developed to reconstruct 3-D internal images of biomaterials [4]. This 3-D internal structure microscopy was applied to observe the internal structure of several histological biomaterials and agricultural products [1-3,5].

The objectives of the study were to develop a novel technique to observe and reconstruct 3-D images of ice crystals and to investigate the effects of freezing conditions on ice crystal size, morphology, volume and distribution of the ice crystals formed in frozen dilute solution.

## MATERIALS AND METHODS

Two types of freezers [6] were prepared for accomplishing one-dimensional freezing of the samples. A program freezer (TNP87S, Nihon freezer, Japan) is composed of a freezer, temperature controller, liquid nitrogen container and a thermo-recorder. A copper cooling plate was located on the top of the freezer and its surface temperature $T_{cp}$ was controllable by regulating the electric power of a heater contained in the freezer and a flow rate regulator of liquid nitrogen in the range of room temperature to -150℃. For more rapid freezing, similar liquid nitrogen freezer was employed and its cooling plate temperature was controlled at -190℃. The sample holder was placed on the copper cooling plate attached to the freezer. The holder is a cylindrical paraffin vessel of 8mm in diameter and 30mm in height and surrounded by heat insulator for accomplishing one-dimensional freezing. Temperature distribution of the sample was measured by using needle-type thermocouple probes of 0.3mm in diameter made of copper-constantan wires and the changes in temperatures were monitored with a data acquisition system (MV100 Thermo recorder, Yokogawa Electric Co., Japan).

The MSIPS [1-4,6] is composed of a multi-slicing section to expose the cross-sections of a sample, an observation section and an imaging section as shown in Fig.1. The cross-sectional images of exposed surfaces after slicing were captured directly with a CCD camera (DX930, Sony, Japan) through a fluorescent microscope (BX-FLA, Olympus, Japan), and recorded into a laser videodisc (LVR-300AN, Sony, Japan). The multi-slicing section was maintained at -40℃ with an immersion cooler during observation [2,6].

To prepare sample solution, fluorescent Rhodamine B ($C_{28}H_{31}ClN_2O_3$) and agar were dissolved into hot distilled water. Final solute contents of Rhodamine B and agar were in the range of 0.02-0.1wt% and 0.5-1.0wt%, respectively.

The copper column located at the lower part of the holder was used to determine the heat flux across the bottom sample surface during freezing. The solution was poured onto a copper column in the sample holder. When the sample was cooled down to room temperature, it was located on the copper cooling plate of freezer. The temperature of the copper cooling plate was controlled according the freezing condition for each sample, and then

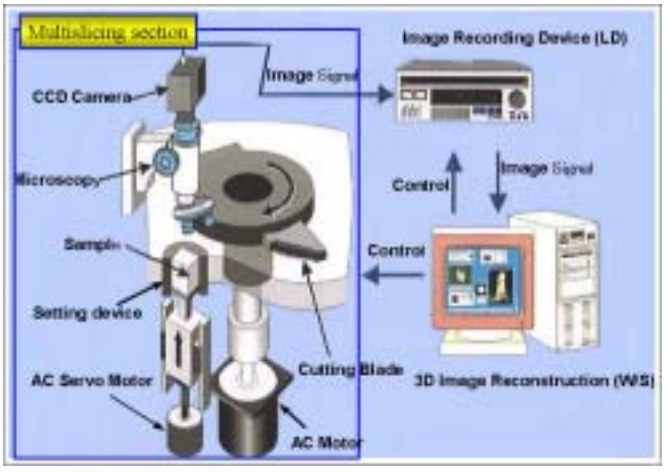


Fig.1. Micro-slicer image processing system (MSIPS).

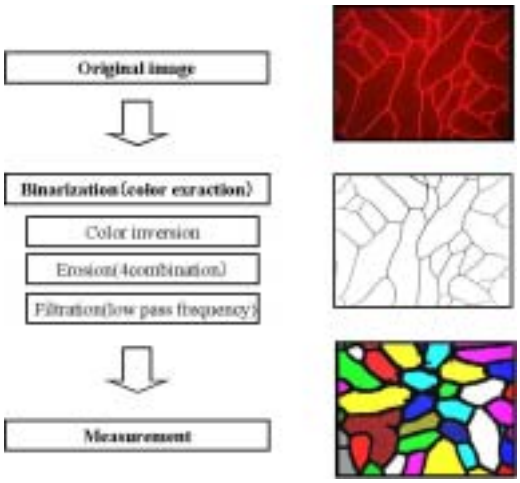


Fig.2. Flow diagram of image analysis

one-dimensional freezing was carried out. After freezing, the sample holder was moved into the setting device of the MSIPS. The sample was continuously pushed up by an AC servo motor and sliced together with the paraffin sample holder at the revolution rate of 60rpm with the thickness of 5μm. The images of 3600 cross-sections per sample were obtained serially. The recorded 2-D images had 256 gray level with the resolution of 640pixels x 480pixels (1pixel = 1.1μm). The 3-D image was reconstructed based on a volume rendering method by utilizing 3-D visualization software (AVS Express, Advanced Visual System Inc., USA) and displayed the internal structure as well as an arbitrary cross section of the sample choosing observation angles.

The morphology of ice crystals was analyzed by the image analysis software (SPICCA2 TVIP-5100, Nippon Avionics, Japan). The procedure of image analysis is shown as a flow diagram in Fig.2. Captured images were binarized and modified by means of inversion, erosion and low pass frequency filtration. Each of the ice crystals is individually labeled and their characteristic parameters were analyzed. The major length (*ML*), minor width (*MW*) and the equivalent diameter of ice crystal area (*d*) were measured, while their ratio (*ML*/*MW*) was determined as an index of morphology and a tortuosity factor (τ) was calculated as the square of the ratio of the effective vertical length of ice column *(Le*) to the thickness of an image (*L*). The volumes of ice columns were also determined by a commercial volume analysis software (TRI 3D volume, Ratoc System Engineering Co. Ltd., Japan).

## 3. RESULTS AND DISCUSSION

In this section, the typical results obtained are presented for the samples having solute contents of 0.1wt% Rhodamine B and 1.0wt% of agar, respectively.

Fig.3 shows the horizontal and vertical cross-sectional images of ice crystals at *Tcp* -30℃, respectively. These images clearly show the ice crystals and concentrated Rhodamine B; the former was distinguished by dark color, while the latter turns out white. These results demonstrated that MSIPS allowed to observation of ice crystals directly in a frozen dilute solution.

A series of 200 cross-sectional images of 1mm height was analyzed to investigate the distribution of ice crystal size. Fig.4 shows a relative cumulative distribution of the equivalent diameters of ice crystals at -40℃. These

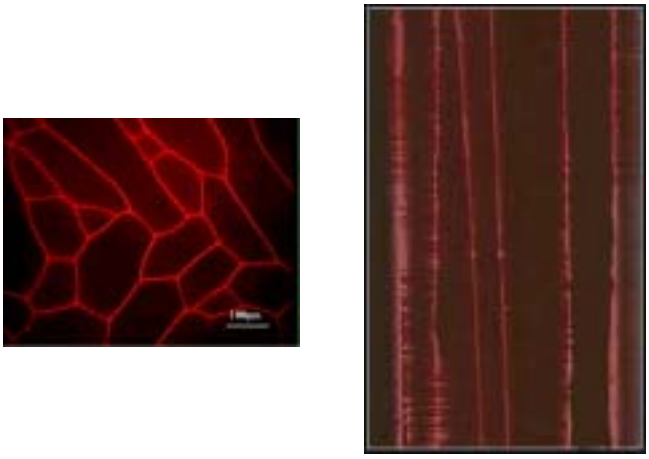

Fig.3. Horizontal and vertical cross-sectional image of ice crystals at *Tcp* －30 ℃

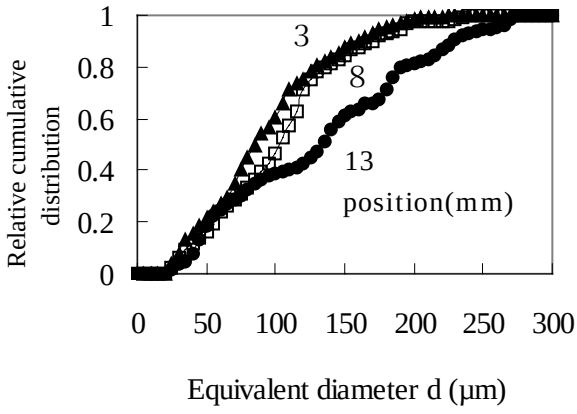


Fig.4. Relative cumulative distribution of ice crystal diameter at *Tcp* －40 ℃

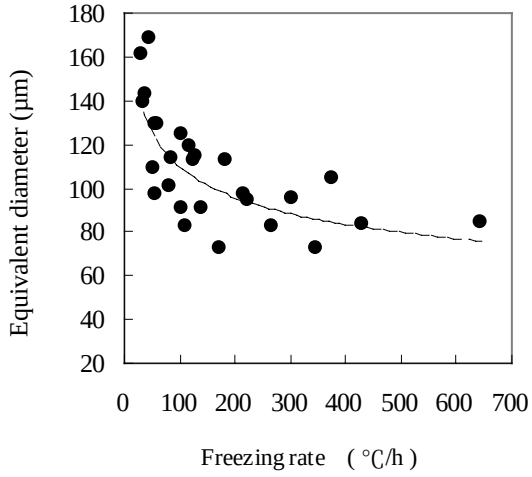


Fig.5. Ice crystal diameter according to the freezing rate. Experimental data:(●) and fitting curve (-).

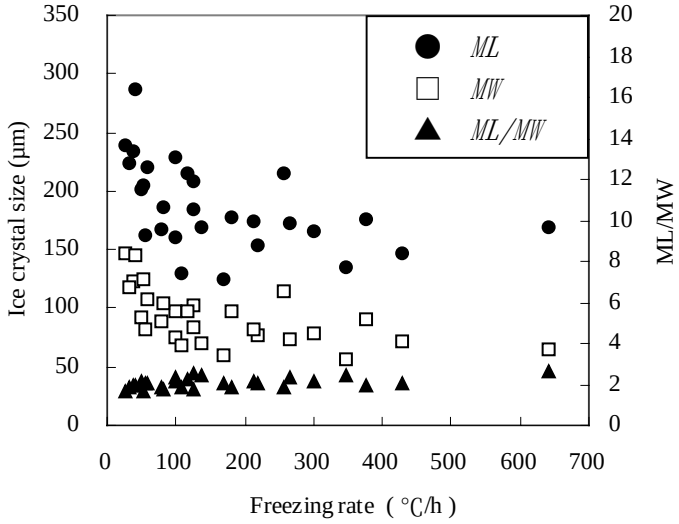


Fig.6. The effect of the freezing rate on ice crystal size and *ML/MW*.

curves were obtained at the locations of 3mm, 8 mm and 13mm from the bottom surface, and the number of ice crystals counted were 3667, 2492, and 1497, respectively. The equivalent diameters were increased according to the distance of the ice crystals from the bottom surface.

The equivalent diameter according to freezing rate is plotted in Fig.5, which was obtained by pooling all the results. The equivalent diameter of ice crystals were in the range of 85μm to 169μm, and then decreased exponentially in increasing freezing rate at *Tcp* -20℃ to -80℃. The ratio of mean major length (*ML*) to mean minor width (*MW*) was calculated for obtaining a quantitative index of morphology. As the ratio approaches close to unity, morphology can be regarded as circular or cubic. On the other hand, it would be ellipse or rectangular, if the ratio was far different from unity. As shown in Fig.6 the values of ML and MW also decreased in increasing the freezing rate and the ratio (*ML/MW*) was in the range of 2.0 ± 0.5, indicating no significant dependence of freezing rate on ice crystal morphology.

The effective vertical lengths were measured from vertical cross-section images and tortuosity factor was calculated to 1.0134 at *Tcp* -40℃.

Figure 7 shows the 3-D ice crystal morphology at *Tcp* -120℃. The scales of the image were 678μm in length, 473μm in width and 1000μm in height, respectively. An arbitrary cross-section of ice crystal is also shown in Fig.8. Figure 9 shows a 3-D image of the labeled ice columns and three extracted ice columns at 8mm from the

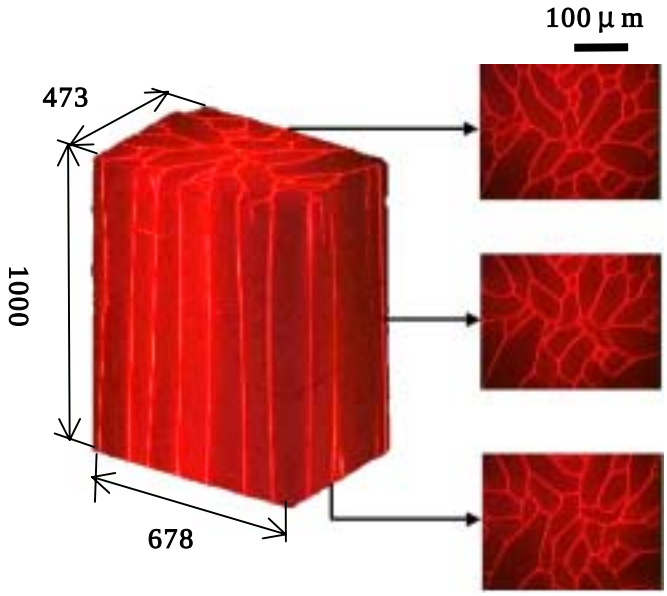


Fig.7. 3-D image of ice crystal at *Tcp* -120℃

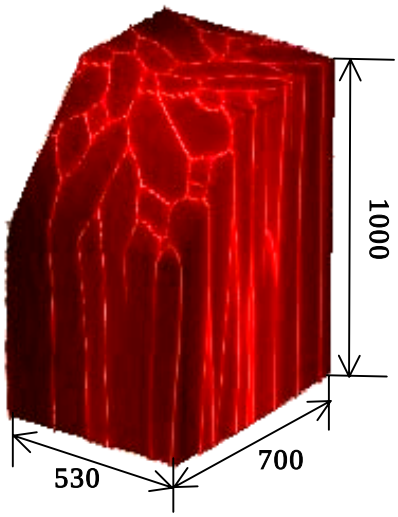


Fig.8. Arbitrary cross-section of 3-D ice crystals at *Tcp* -120℃

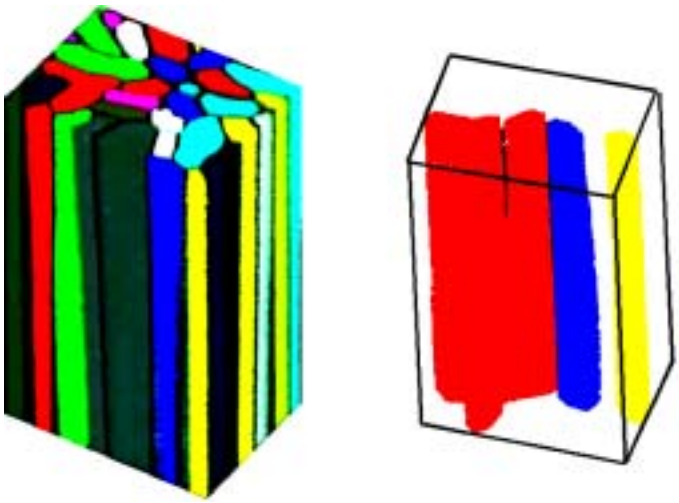

Fig.9. 3D image of labeled ice columns at 8mm from cooling surface (at *Tcp* -40℃)

cooling plate at *Tcp* -40℃. The scales of the image were 700 μm in length, 530μm in width and 1000μm in height, respectively.

The 3-D morphology of ice crystals was found to form a bundle of continuous columns or dendrite columns at any freezing conditions. The volume distribution of the ice columns at *Tcp* -40℃ was calculated from labeled ice columns shown in Fig.11. The ice columns summed up to 2.6 x $10^8$μm$^3$, which was 69% of the whole observed volume, 3.7 x $10^8$μm$^3$. As shown in Fig.12, the volume of individual ice crystals were in the range of 4.6 x $10^4$μm$^3$ to 3.3 x $10^7$μm$^3$, and 36 ice columns were counted in the 3-D image.

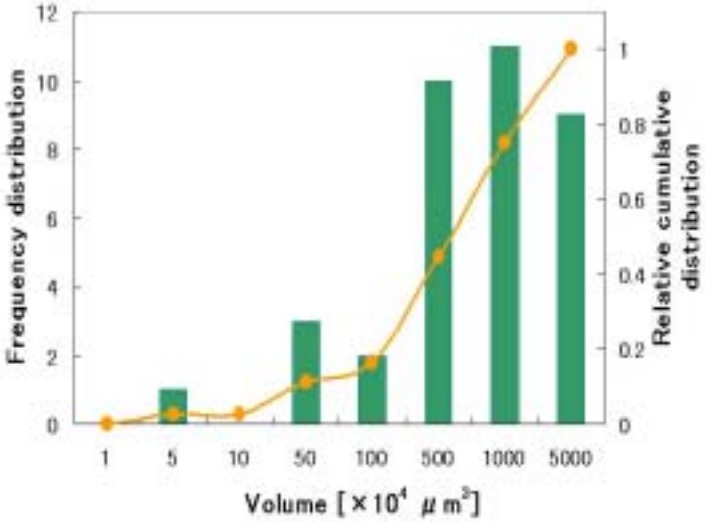


Fig.10. The volume distribution of ice columns (at *Tcp* -40℃)

The proposed method using the MSIPS has demonstrated various advantages compared with conventional observation techniques and also provided a new tool to investigate quantitatively the relationships between freezing conditions and characteristics of ice crystals such as morphology, size and distribution.

## CONCLUSION

The 3-D morphology and distribution of ice crystals in a frozen dilute solution with agar was quantitatively analyzed with the Micro-Slicer Image Processing System (MSIPS).

## NOMENCLATURE

| | | | |
|---|---|---|---|
| $T_{cp}$ | copper cooling plate temperature, ℃ | $L$ | vertical length of ice column, μm |
| $R$ | freezing rate, ℃ $h^{-1}$ | $Le$ | effective vertical length of ice column, μm |
| $d$ | ice crystal diameter, μm | $q$ | heat flux, J $m^{-2}$ s |
| $ML$ | ice crystal major length (major axis), μm | $V$ | volume, $\mu m^3$ |
| $MW$ | ice crystal minor width (minor axis), μm | τ | tortuosity factor, - |

## REFERENCES


[1] Do G., Sagara Y, Kudoh K, Yokota H and Higuchi T. 1997, Surface area and volume measurements of a Broccoli (Brassica oleracea L.var.italica PLEN.) with a Microslicer-Image Data Processing System (in Japanese). *J. society of Agr. structures*, 28:p.21-29.

[2] Do G., Sagara Y., Tabata M., Kudoh K. and Higuchi T., 2003, Three-dimensional measurement of ice crystals in frozen beef with micro-slicer image processing system. *Int J.Refrigeration* (in press)

[3] Kimura J, Tsukise A, Yokota H, Nambo Y, Higuchi T., 2001, The application of three-dimensional internal structure microscopy in the observation of mare ovary. *Anatomia histrogia embryologia*, 30:p.309-312.

[4] Kobayashi K, Higuchi T, Aoki I, Kudoh K., 1995, Development of microslicer implementation of 3-dimensional internal structure (in Japanese). *J. Japan society for precision eng*., 61:p.100-104.

[5] Ogawa Y., Sugiyama J., Kuensting H., Ohtani T., Hagiwara S., Liu X. Kokubo M., Kudoh K. and Higuchi T., 2001, Advanced techinique for three –dimensional visualization of compound distribution in a rice kernel. *J Agr. Food Chem*..49:p.736-740

[6] Ueno.S., Do G., Sagara Y., Tabata M., Kudoh K. and Higuchi T., 2003, Three-dimensional measurement of ice crystals in frozen dilute solution. *Int J.Refrigeration* (in press)