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Applications of gray-level variation detection method to intracellular ice formation

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ABSTRACT

Intracellular ice formation (IIF) is the major cause of death in cells subjected to freezing. The occurrence of intracellular ice prevents the penetration of light into the camera and makes the image dark. Therefore, the gray-level variation can reflect the IIF. However, cell deformation is accompanied with IIF, especially for larger cells. It is necessary to account this entire phenomenon together in a single method. In this paper, the normalized parameter C defined by the gray-level variation depending on the displacement was defined to reflect the gray-level change of each pixel point in the region of interest of the image. The process of IIF of onion epidermal cells and 293T cells was analyzed by this method.

1. Introduction

Intracellular ice formation (IIF) is the major cause of death in cells subjected to freezing because of its destruction to the subcellular structure and the cell membrane [1,11,17]. For the fatal effect of IIF on cryopreservation, considerable works have investigated the effect of IIF on the survival rate or level of injury to the cells [17,22]. In these experiments, IIF is characterized by "sudden darkening," which means the very rapid growth of ice in the cell. However, for the rapid growth of ice, only a high-speed video can discern the intracellular crystallization process as a single advancing solidification front within each cell.

Yang et al. observed the initial site of IIF in human umbilical vein endothelial cells [18] and MCF-7 cancer cells [19] by using a high-speed camera at a frame rate of 1000fps. Li [9] analyzed the mechanism of IIF by observing the repetition of freezing and thawing using a high-speed camera of 1000fps. However, the frame rate increases at the expense of decreased resolution. Ninagawa [13] captured the intracellular freezing in plant cells at a maximum frame rate of 2000 fps and a resolution of 512 × 512 pixels. Stott et al. [15]described the first observations and measurements based on cryomicroscopy experiments recorded at a frame rate of up to 16000fps with a resolution of 256 × 128 pixels.

In most of the above-mentioned papers, the motion of intracellular crystallization fronts was detected by frame-by-frame playback of high-speed video recordings. However, because of the large number of the images produced by a high-speed camera, the manual observation or detection is considered very cumbersome and a time-consuming task and prone to human errors [6].

The visualization of the motion of the IIF depends on the fact that the region of ice is darker in the images, which means the appearance of higher gray level pixels in the image. The use of an effective way to distinguish the gray-level variation may improve the detectability of IIF. Gray-level tracking methods based on photogrammetric and image processing have expanded over the last decade attracting researchers from the fields that include medicine [6,10,20] and materials [21]. The gray level from images can be easily extracted by many softwares. However, the gray-level tracking method is seldom used for ice formation analysis, which is complicated for many reasons. Weak image contrast, significant shape deformations of cells, and the drift of cells caused by extracellular ice together make such a tracing procedure quite a difficult task [16].

The objective of this paper is to present an efficient and effective intracellular ice front tracking method according to the gray-level variation of the image sequence, thereby relieving the burden of manual work.

2. Method

2.1. Preparation of cell images

Epidermal tissue of fresh onion was used as specimens. Fresh onions of the same maturity without any disease and damage were bought from the market. Each specimen was cut into a 10 mm^2 section and placed onto a cover glass with distilled water to prevent it from drying. A second cover glass was then placed over the specimen. The cover glasses were placed on the cooling stage and cooled from room

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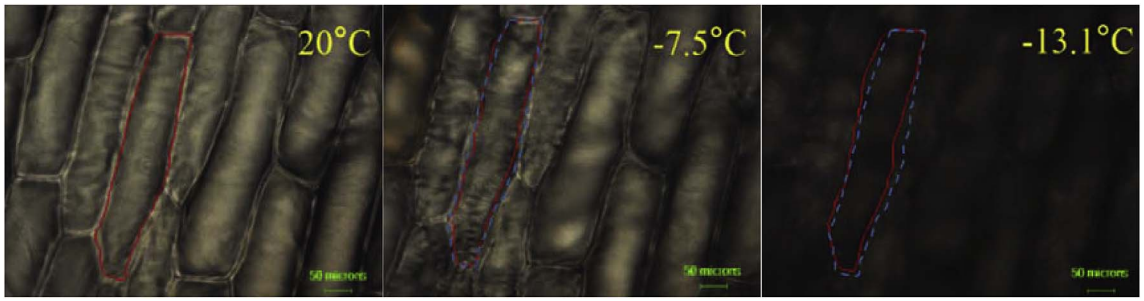

Fig. 1. Shape variation of the cells during IIF.

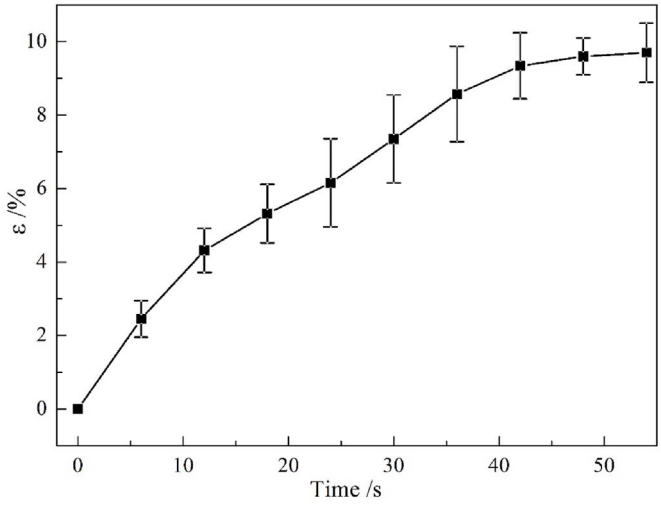

Fig. 2. Degree of cell deformation during IIF.

temperature to the temperature at which intracellular freezing occurred at a cooling rate of −10 °C/min.

The cryomicroscopy system used in this study consisted of a microscope (Olympus BX51, Japan) equipped with BCS-196 freeze stage (Linkam Scientific Instruments, Tadworth, Surrey, UK) and a high-speed camera. To track the rapidly advancing solidification fronts, a frame rate of 2000 frames/s was employed, which necessitated high level of illumination of the sample and a resolution of 512 × 512 pixels. Adequate lighting could be achieved by using a standard 100 W mercury-vapor lamp. A mercury lamp is more energy efficient, which can supply more light than a halogen lamp. To maximize illumination, all other filters and inserts were removed from the light path. Termination of video acquisition was triggered when any of the conventional indicators of IIF were observed. In each experiment, only one cell or two with IIF can be recorded because of the limited random access memory of the camera.

2.2. Cell deformation during IIF

During the experiment, dynamic changes in the appearance and structure of onion epidermal cells were observed at a cooling rate of −10 °C/min using the cryomicroscope. The cell that underwent IIF was marked in the image, and the shape variation of the cells is illustrated in Fig. 1. It can be found that as the temperature dropped, the ice crystals formed progressively in the cells and the image became dark and blurring. During this process, the cell was out of its original shape and location after IIF. There were three reasons for the deformation of cells during IIF: (1)transmembrane water transportation due to the osmotic pressure in the outer cells, (2)the influence of extracellular ice and other cells, and (3)the expansion of water during freezing.

Fig. 2 shows the degree of cell deformation (ε), which indicated how much a cell had been deformed, and it was measured by calculating the area of cell before (A_b) and immediately after (A_c) intracellular freezing. ε was then calculated as follows [12]:

$$\varepsilon = \frac{A_c - A_b}{A_b} * \% \tag{1}$$

From Fig. 2, it is obvious that ε increased with time. Finally, the degree of cell deformation reached at about 9.5% after the completion of IIF. This indicated that as the gray level varied, the deformation was accompanied with ice formation process. It is important to account this entire phenomenon together in a single method.

2.3. Gray-level variation detection methods

In the image sequences of a cell undergoing IIF, the dark pixels correspond to intracellular ice crystals and the bright pixels to the

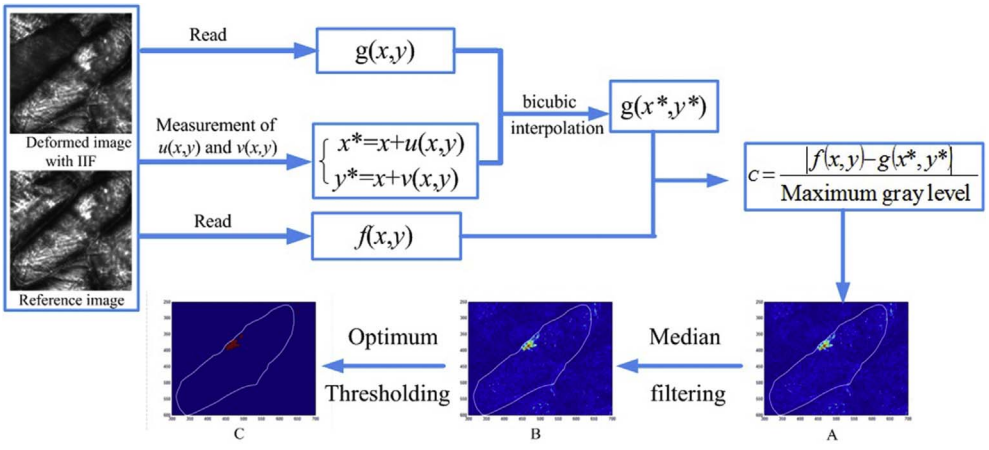

Fig. 3. Flow chart of gray level variation detection methods.