Micro-scale thermograhy of freezing biological cells in view of cryo-preservation

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Abstract

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The direct view of latent heat released from the inter- and intra-cellular ice formation during freezing of biological living cells and tissues are obtained by micro-scale IR thermography. The phenomena observed during freezing, such as a latent heat generation, an explosion of a cell wall and the vitrification of cytoplasm, with and without cryo-protective agents are discussed in relation with the cryo-preserving technique. A theoretical and numerical modelling of inverse problem applied to explode and diffuse of latent heat is discussed to determine thermal diffusivity and thermal heat source related to latent heat. The latent heat generation of cells becomes indistinct and slow going but follows a repeated freezing in the cryo-protective solvents.

1. Introduction

The principles of cell damage during cryo-preservation are analyzed as the solution effects, extra cellular ice formation, dehydration, and intracellular ice formation. However, the freezable cells and tissues are still limited. Vitrification by adding a cryo-protectant agent to the biological cell systems has been proposed as a means by which the structural and functional integrity to be maintained with a recovered viability after freezing. The minimum concentration to avoid toxic effect and the well-controlled freezing/thawing rate must be examined for each system. To quantify the cell death has been executed by staining cells for the fluorescent microscopy with the result of a biological assay. Until recently the direct view of latent heat of inter- and intra-cellular ice formation and thermal diffusion over the tissues[1] had not been executed. By using a high-speed IR camera with a micro-scale spatial resolution the latent heat explosion and diffusion over the tissues were clearly visualized. This study gives more quantitative analysis of freezing phenomena of biological cells with a theoretical and numerical modelling of inverse problems applied to explode and diffuse of latent heat.

2. Experimental

2.1. IR thermography

High-speed IR FPA system, Phoenix (Indigo) and Radiance HS (Raytheon), having an indium-antimony (InSb) sensor array of 320x256, or 256x256 pixels with the optimum wavelength between 3μm and 5μm was used for the measurement. The frame rate for taking image was selected 250 ~1000 frames/s (4msec~1msec per one picture) in this study. The direct access to Hard disk was originally prepared for the longer time data storage with the high speed. By originally designed silicon germanium made microscopic lens, the area of 1.9mmx 1.9mm corresponding to the spatial resolution of 3.0 μm x3.0 μm for each pixel is visualized. The length and distortion are calibrated by using a standard micro-scale of USAF 1951. The intensity is calibrated with the temperature sensor by using the certified reference sample.

2.2 Thermal diffusivity

Thermal diffusivity was measured in situ by temperature wave analysis method during the freezing and thawing of the biological cell systems and tissues.

2.3 Biological cell systems

Onionskin cell was used as a typical plant cellular tissue linked and separated with the cell wall. A cultured tobacco cell in the medium was used as a typical example for the cryo-preservation. Tobacco cells are one-dimensionally connected with each other.

3. Analytical procedure

The numerical analysis of 2D temperature processing is based on the method of C. Pradre et al[2]. The 2D finite differences approximation of the previous expression by considering the non calibrated temperature signal $T_{i,j}^{k}$ of one pixel at node *i,j* and at time *k* is such as:

$$\left(T_{\mathrm{i+1,j}}^{k}+T_{\mathrm{i-1,j}}^{k}+T_{\mathrm{i,j+1}}^{k}+T_{\mathrm{i,j-1}}^{k}-4\,T_{i,j}^{k}\right)Fo_{i,j}^{k}+\Theta_{i,j}^{k}=T_{i,j}^{k+1}-T_{i,j}^{k} \qquad (1)$$

where Δx: pixel size (*m*), Δt: time step (*s*) and $\Theta_{i,j}^{k}$ is a local source term

$$Fo_{i,j}^{k}\Delta T_{i,j}^{k}+\Theta_{i,j}^{k}=\delta T_{i,j}^{k} \qquad (2)$$

with $\Delta T_{i,j}^{k} = \left(T_{i+1,j}^{k} + T_{i-1,j}^{k} + T_{i,j+1}^{k} + T_{i,j-1}^{k} - 4\,T_{i,j}^{k} \right)$, $\delta T_{i,j}^{k} = T_{i,j}^{k+1} - T_{i,j}^{k}$ and $Fo_{i,j}^{k}$ is a non dimensional Fourier local number such as

$$Fo_{i,j}^{k} = \frac{a_{i,j}^{k} \Delta t}{\Delta x^{2}} \qquad (3)$$

where $a_{i,j}^{k}$ is the apparent local thermal diffusivity. With the inversion matrix procedure both parameters of $Fo_{i,j}^{k}$ and $\Theta_{i,j}^{k}$ are determined.

4. Results and discussion

Figure 1 shows the morphological change of onionskin cells in the repeated freezing and thawing processes observed under the optical microscope. After 1st freezing the cell wall was broken and in the 2nd freezing thermal diffusivity increases.

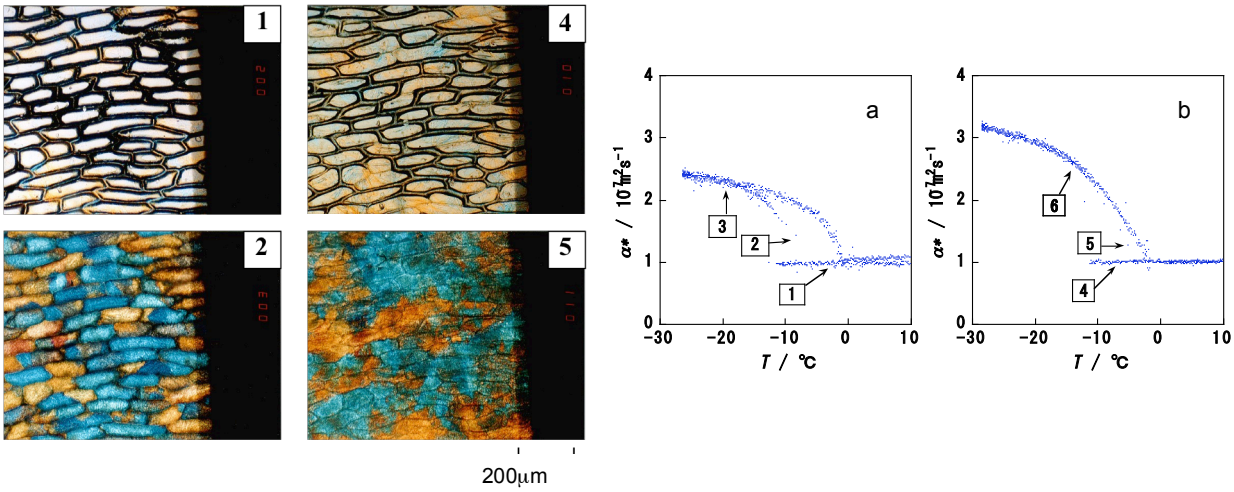

Fig. 1. *Freezing onionskin cells observed under the polarized optical microscope (1) before freezing, (2) in 1st freezing, (4) after thawing, (5) in 2nd freezing, (a) thermal diffusivity ($\alpha*$) in 1st freezing and thawing, (b) thermal diffusivity in 2nd freezing and thawing.*

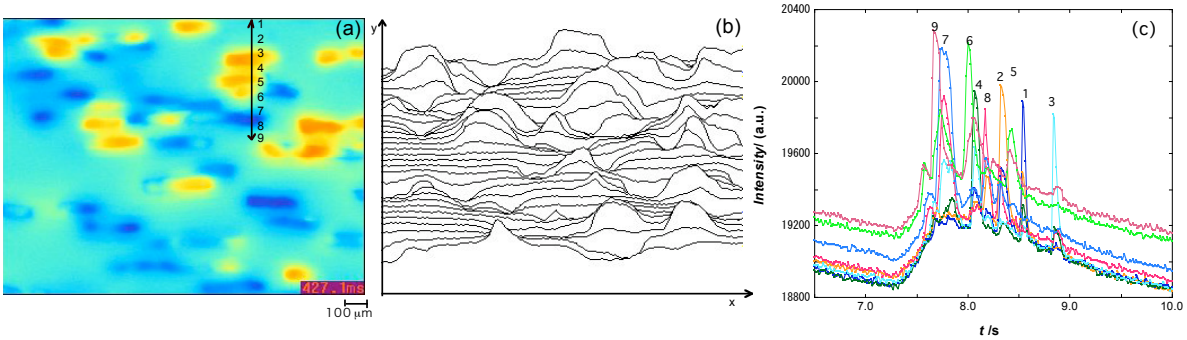

Fig. 2. *Differential coefficient images (with time) of freezing onionskin cells, (a) a colored view, (the bright color corresponds to a positive coefficient and the dark color corresponds to a negative one, (b) a bird's-eyed view, (c) two- dimensional micro-scale thermal analysis of each single cell as numbered in (a).*

Figure 2 shows the time differential coefficient images of the latent heat generation on freezing. By analyzing the micro-scale 2D thermal analysis curves in Fig. 2c it is proved that the temperature field generated by latent heat influences the freezing phenomena itself. The local temperature field dissipation will be given by the inversion analysis.

REFERENCES

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