http://dx.doi.org/10.21611/qirt.2006.036

Two-dimensional thermal analysis of organic materials by IR thermography

by J. Morikawa*, T. Hashimoto*, E. Hayakawa**, T. Eto***,

*Tokyo Institute of Technology, Tokyo, Japan
**ai-Phase, Co., Ltd., Tokyo, Japan
***Eto Denki, Co., Ltd., Tokyo, Japan

Abstract

Two-dimensional micro scale thermal analysis is proposed for the measurement of the latent heat released from the biological cells during freezing by use of the high-speed infrared focal plane arrays. The differential thermo graphical image of latent heat spreading over the tissues and the inter- or intracellular thermal diffusion are detected with a spatial resolution of 3~7.5μm. The temperature distribution in time and space of each biological cell is influenced by both the self-release and the intercellular diffusion of latent heat. This method is also applied to the phase transitions of organic materials.

1. Introduction

Thermal analysis is the analytical method to determine thermal/physical properties as a function of temperature. Differential scanning calorimetry is the typical one that is used to determine the heat capacity and the enthalpy during the phase transitions. It is one of the essential methods in thermal science and technology, not only in the material science but also in the pharmacy and bioscience. However, the averaged thermal property taken by this method often does not bring the sufficient information when the small-scale distribution exists.

In this study two-dimensional micro scale thermal analysis by the direct measurement of latent heat released from the organic materials and biological cells during phase transitions is proposed by use of the high-speed infrared focal plane arrays. The differential image of thermo graphically observed latent heat spreading over the homogeneous / inhomogeneous structures of the materials allow estimating the two-dimensional distribution of the thermal properties. The influence of the inter-cellular and intra-cellular thermal diffusion of the released latent heat in the freezing of biological tissues is presented in the single cell scale.

The cryo-preservation of cultured cells is an indispensable technique in biological engineering. The control of intracellular ice formation or glass formation, and dehydration with an extra cellular solution are important for cryo-preservation, because biological cells are easily damaged by the volume change of ice formation. According to a typical thermodynamic rule, the 1st-order phase transition is accompanied by not only a volume change but also latent heat. However, there are few thermo graphical studies applied to the thermal analysis of biological cells on the unit cell scale for measuring the latent heat release and the thermal diffusion during freezing.

http://dx.doi.org/10.21611/qirt.2006.036

2. Experimental

2.1. IR FPA system

The apparatus of measuring system consists of a specimen holder in a vacuum chamber, a temperature control system, an IR camera, a microscopic lens with a magnification of x 10 and a personal computer. The specimen is directly put on the thin gold plate (with thickness 100 μm) fixed on a Peltier chip with a heat sink in the vessel. The specimen holder was controlled at a constant cooling rate 80°C/min from room temperature to –20°C. To avoid condensation of water on a specimen surface the atmosphere in the vessel is filled with dried nitrogen gas. The observation was carried out through a sapphire glass window.

High-speed IR FPA system, Phoenix (Indigo) or 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 7.5μmx7.5μm 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 Biological cells

Onionskin cell was used as a typical plant cellular tissue linked and separated with the cell wall, which was cut into 10mm x 10mm area size from the inner side of the second stratum from the outer side. The averaged cell size is about 80 μm x 200μm in area size and 80μm in thickness. (See Fig.5, the IR image of onionskin cells at around 0°C).

A cultured tobacco cell in the culture medium was used as a typical example for the cryo-preservation. Tobacco cells are one-dimensionally connected with each other. (See Fig. 6 with the IR image at room temperature in the medium).

3. Analytical method

To detect a small temperature change generated by the small amount of latent heat released from a single cell during the phase transition at sub-zero temperature, the imaging transform procedures are prepared such as;

i) The draw of differential images calculated by the equation

$$\mathrm{I}'(\mathrm{t}) = \frac{\partial I}{\partial t} = \lim_{\Delta t \to 0} \frac{\mathrm{I}(\mathrm{t}) - \mathrm{I}(\mathrm{t} - \Delta t)}{\Delta t}$$

Where I is the intensity of each pixel, t is time and Δt is a time interval that can be optimized depending on the rate of the phenomena.

http://dx.doi.org/10.21611/qirt.2006.036

ii) The three-dimensional-draw (x, y, t) of differential image, to extract the spatial temperature gradient in a micro scale.

4. Results and discussions

4.1.1 Freezing of onionskin cells: Differential image

Fig.1 shows a time development of differential thermal image of onionskin cells during freezing. The cooling rate is -20^{o}C/min and the freezing phenomenon is observed at around -9^{o}C. The colour corresponds to the differential coefficient of temperature with time at each pixel size. The bright colour corresponds to the positive coefficient, the dark colour the negative, and the pale blue equals to zero. In Fig.1 the latent heat released from the two adjacent cells in the tissues is captured one by one with some time intervals. It is clarified that the latent heat release starts at a pixel on the cell wall on one side and the self-heat generation successively continues inside the cell, but it stops when the travelling front runs into the other side of the cell wall. At the same time the thermal seeping out is observed as graphically shown in the pale bright colour just around the neighbourhood of the cell wall. This pale colour area is produced by the thermal conduction of the latent heat generated in the adjacent cell propagating via the cell wall. The dark colour of the negative differential coefficient comes from along the cell wall. These phenomena suggest the thermally important role of the cell wall having the characteristic thermal property and the thermal interface between the adjacent cells.

Fig.2 is the 3D plot of the differential image of the freezing onionskin cell in Fig.1. The height corresponds to the differential temperature coefficient with time, positive and negative, and the spatial slope of the coefficient is drawn as an air view. It clarifies the move of the heat front with the spatial gradient of the differential coefficient. When the heat front reaches the cell wall the negative coefficient is observed.

As reported in Ref [1] the thermal seeping out from the adjacent cell has an effect on the freezing of the neighbouring cells. During when the temperature rises up by the heat transfer from the neighbouring cells the freezing is not going on. After the negative coefficient is clearly observed, corresponding to the dissipation of the external heat flow, then the freezing occurs with the latent heat releasing. This procedure is clarified by introducing the differential imaging.

4.1.2 Freezing of culture tobacco cells in the medium

The time evolution of the latent heat release and spread in freezing of the tobacco cells in the medium is shown in Fig. 3 with the differential imaging method. In cooling the traveling front of the generating latent heat of the medium solution is first observed. After the negative coefficient (dark color) occurs and disappears, then the flickering of the bright and dark spot with the area size of smaller than 100μm in radius is observed in a repetition. The freezing starts at the sub-zero temperature and the flickering time of the bright and dark is less than 2ms. Tobacco cells are

http://dx.doi.org/10.21611/qirt.2006.036

linked one-dimensionally via the cell wall each. The freezing occurs cell-by-cell separated by the cell wall, but in this case the different rule of the dissipation of the latent heat can be observed. The continuous flickering of the bright and dark color, the positive and the negative coefficient, in the adjacent linked cells suggests the succeeding freezing of the adjacent tobacco cells. It might be resulted in the surrounding thermal environment. After freezing of the medium solution the tobacco cells are surrounded by the frozen ice having the eight times higher thermal diffusivity than the unfrozen cells. The latent heat can dissipate to the direction of the ice contact and the seeping out of thermal flow is not serious than observed in onionskin cells. With the certified temperature calibration the temperature rise at the center of each cell is estimated as 1.1^{o}C. This small temperature change on freezing can be clarified by this differential imaging method for the first time.

Fig. 4 is the 3D plot of the differential image of the freezing tobacco cells in the medium that is shown in Fig.3. Precisely the positive and the negative spatial slope are observed corresponding to the flickering of each cell scale. The different spatial slopes of the curvatures observed at the medium or tobacco cells freezing suggest that the influence of the geometry is important.

4.2.1 Freezing of onionskin cells: Micro scale thermal analysis

The identified pixels indicated in IR photo in Fig.5 were chosen for the precise analysis from the view points of the inter-cellular and intra-cellular thermal conduction in the onionskin cells. Fig.5 shows typical temperature profiles on freezing of the neighbouring 5 cells with the pixels in the centre position depicted on the horizontal line as A~E. At a moment of freezing after super cooled to –8.5^{o}C, an obvious temperature rise by crystallization of water in the cell is observed in the characteristic way. It is remarkable that all pixels are cooled uniformly before freezing. When a first temperature step up occurred in the specimen the cooling temperature shifted 3^{o}C higher caused by the freezing of interface water between onion and the base gold plate. This result indicates that the thermal contact between the specimen and the base gold plate is good since all pixels are in the same behaviour before occurring an individual freezing.

In each pixel, characteristic profile is obviously obtained as a superposition of several peaks, one main peak and several smaller side peaks. The highest peak of any pixel results from the latent heat self-generated on the freezing of the cell itself and the amount of temperature rise is almost constant at 5^{o}C. On the other hand, the smaller peaks are caused by the heat conduction from the neighbouring cell, in which a latent heat is generated at a different time on the freezing of the different cell. The peak height of smaller side peak corresponds to the distance from the influential cell. The summation of temperature rise of all pixels corresponds to the exothermic enthalpy in the conventional thermal analysis.

In Fig.5 when cell C starts to freeze, cells A and B are already frozen, on the other hand, cells D and E are still in the super cooled liquid state. A rapid temperature increase is observed at pixel B with a higher thermal diffusivity (α=1.2x10^{-6}m^{2}s^{-1}, determined by temperature wave analysis), but a time- delayed

http://dx.doi.org/10.21611/qirt.2006.036

slower temperature increase is observed at pixel D with a lower thermal diffusivity (α=1.5x10^{-7}m^{2}s^{-1}). The small temperature increases at pixels A and E are also observed with a small influence. The high resolution of time and space of IR FPA elucidates the micro-scale temperature gradient in the onionskin cells caused not only by the latent heat self-generated in freezing but also by the thermal diffusion from the surrounding cells.

4.2.2 Freezing of cultured tobacco cells: Micro scale thermal analysis

Fig.6 shows the time-intensity profile of the linked tobacco cells a~c, and the non-linked cell of d in the IR photo in Fig. 6. The profile shows the large exothermic temperature rise followed by the several small ones. The first peak is attributed to the exothermic latent heat of the medium solution and the small peaks are to the latent heat from the freezing of tobacco cells. In the adjacent linked cells the thermal diffusion of the latent heat is also observed. The freezing of cells a~c are observed by each 10ms and in cell b the temperature rise by the thermal diffusion from cell a and c are observed. On the other hand in the non-linked cell d no influence of the thermal diffusion from the cells a~c are observed. In the freezing process of the tobacco cells the latent heat generation and the thermal diffusion are both observed. To observe the thermal diffusion in the micro scale in addition to the observation of the spatial distribution of the latent heat is the advantage of this method.

5. Conclusion

Micro scale thermal analysis by using IR-FPA is presented applying to the analysis of the freezing phenomena of the biological cells. The high resolution of time and space combined with the time-differential imaging technique clarifies the inter- and intra-cellular heat generation and the heat transfer. The new advantage of IR thermographs to quantify the thermal property and the phase transition is shown as a powerful methodological tool in thermal science.

REFERENCES

[1] T. Hashimoto, and J. Morikawa. Two dimensional micro scale thermal analysis of freezing of onionskin cells by high-speed infrared focal plane arrays, *Jpn. J. Appl. Phys. Physics,* 42 (2003) L706-L708.

[2] J. Morikawa, T. Hashimoto, E. Hayakawa, and H. Uemura. Two-dimensional thermal analysis for freezing of plant and animal cells by high-speed microscopic IR camera, *SPIE*, 5073 (2003) 148-153.

[3] J. Morikawa, T. Hashimoto, K. Yamamoto, and J. Ando. Two-dimensional thermal analysis for freezing of endothelial cells by high-speed microscopic IR focal plane arrays, *SPIE*, 5697 (2005) 282-290.

http://dx.doi.org/10.21611/qirt.2006.036

6. Figures

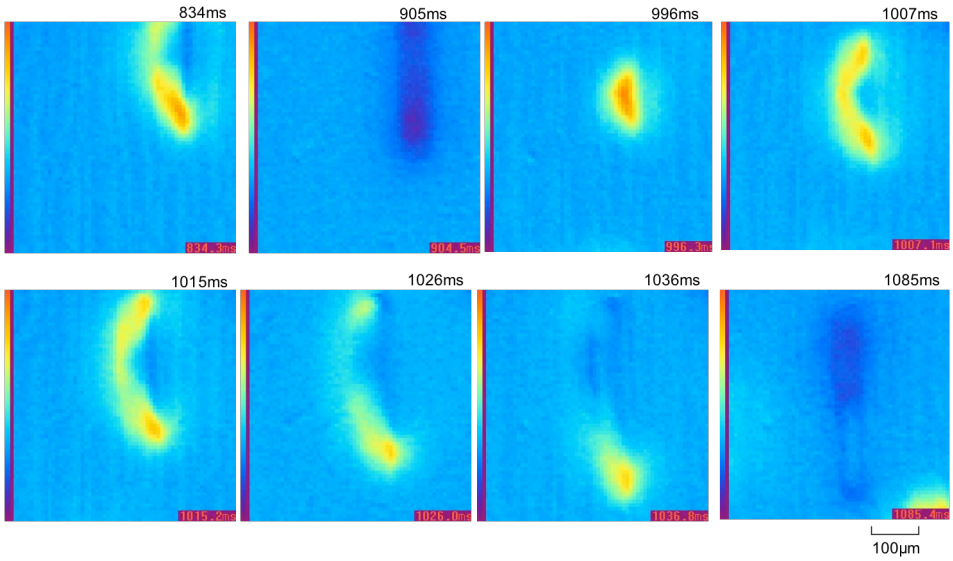

Fig. 1. *Differential image of IR thermographs of the latent heat from a single onionskin cell during freezing.*

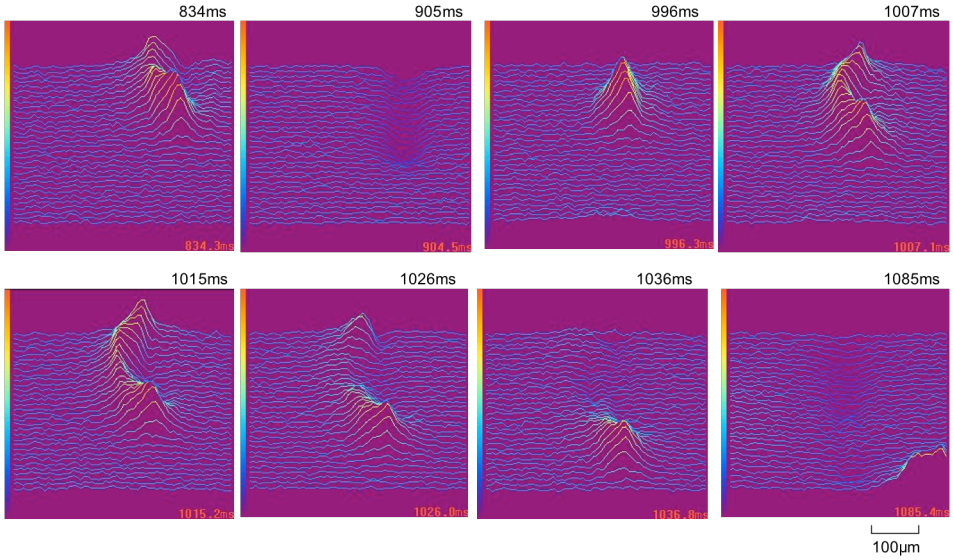

Fig 2. *Three dimensional (3D) plot of differential image of IR thermographs of the latent heat from a single onionskin cell during freezing.*

http://dx.doi.org/10.21611/qirt.2006.036

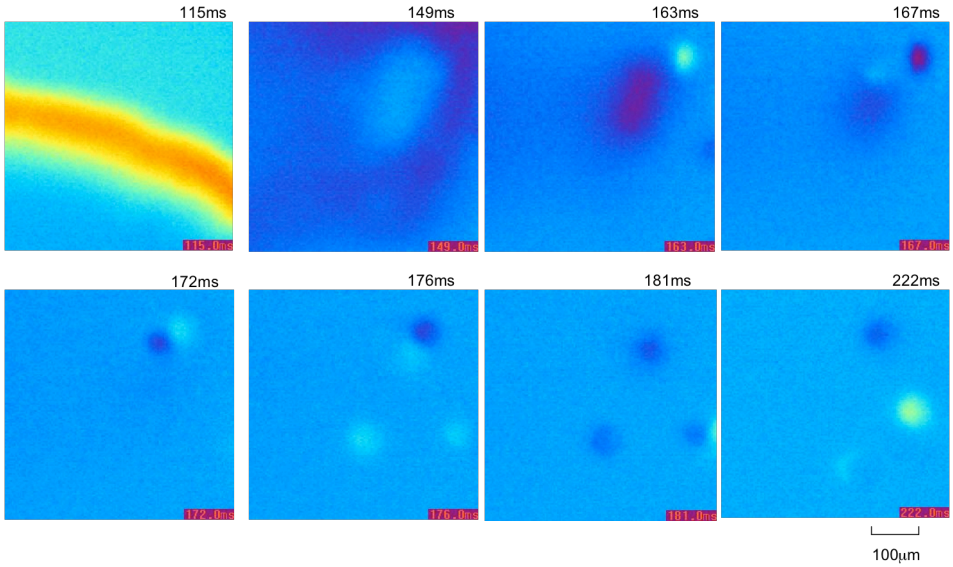

Fig. 3. *Differential image of IR thermographs of the latent heat from a single tobacco cell during freezing.*

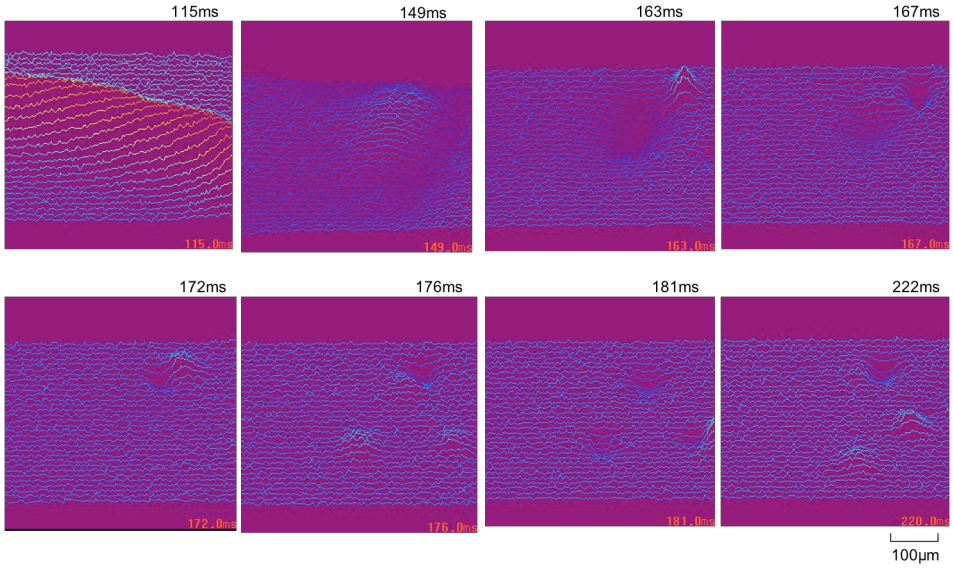

Fig. 4. *Three dimensional (3D) plot of differential image of IR thermographs of the latent heat from a single tobacco cell during freezing.*

http://dx.doi.org/10.21611/qirt.2006.036

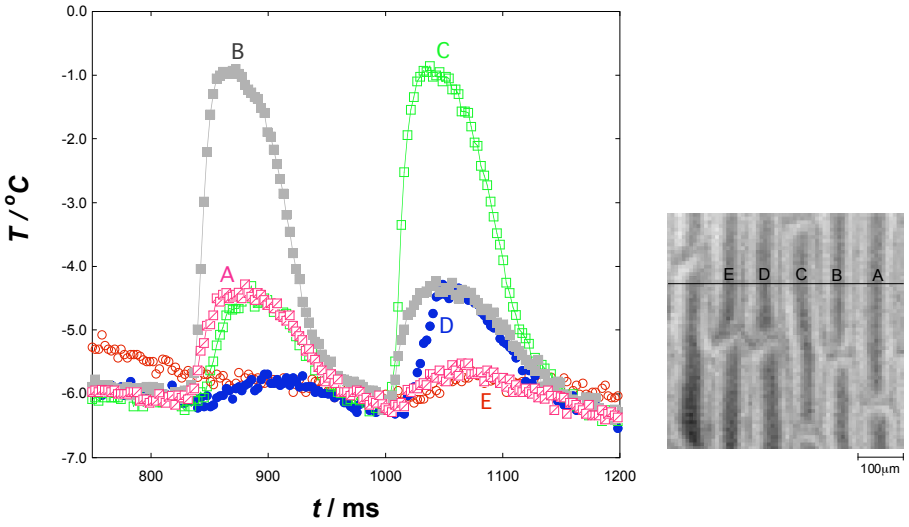

Fig. 5. *Intercellular heat transfer of the latent heat of onionskin cells during freezing affects the temperature-time profile in the individual cells.*

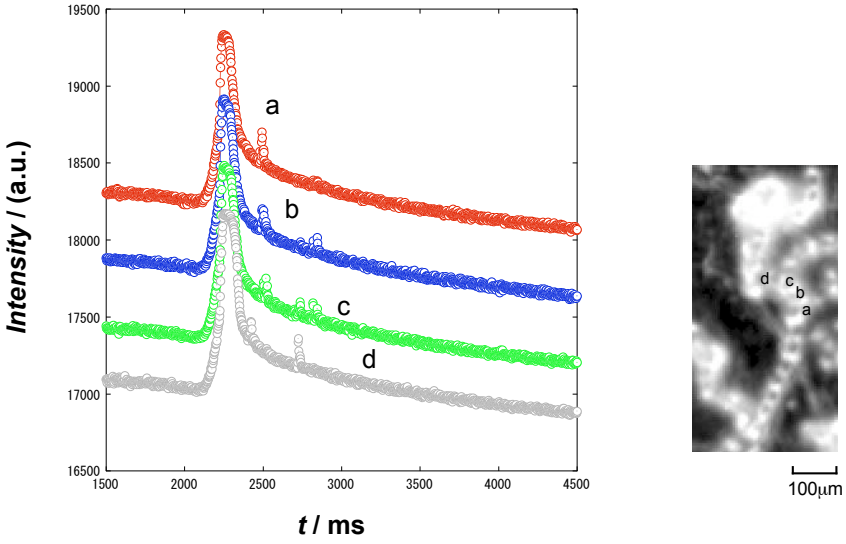

Fig. 6. *Intercellular heat transfer of the latent heat of the cultured tobacco cells during freezing in the medium affects the temperature-time profile of the one-dimensionally linked individual cells.*