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Introductory Words from the Editorial Board

The International Society for Bioelectromagnetism (ISBEM) was founded in 1996 in order to offer an exchange platform for researchers from all over the world regarding advances in bioelectromagnetism. Therefore, the society sponsors the biennially organised international congresses on bioelectromagnetism, starting from 1996. The first International Conference on Bioelectromagnetism (ICBEM) has been held in Tampere, Finland in 1996, After that, the ICBEM conference took place in Melbourne (1998), Bled (2000), Montreal (2002), Minneapolis (2005), Aizu (2007), Rome (2009), Banff (2011), Geneva (2013) and Tallinn (2015). Following the fundamental idea of the ISBEM, the ICBEM provides a platform for researchers all over the world to share their experience regarding their work in the broad field of bioelectromagnetism, which includes:

- The behavior of excitable tissue (the sources)
- The electric currents and potentials in the volume conductor
- The magnetic field at and beyond the body
- The response of excitable cells to electric and magnetic field stimulation
- The intrinsic electric and magnetic properties of the tissue.

This year, we are very happy to welcome all participants to the 11th International Conference on Bioelectromagnetism in Aachen, Germany. In 2018, the ICBEM will be jointly held together with the 13th Russian-German Conference on Biomedical Engineering (RGC) hosted by the Philips Chair for Medical Information Technology (MedIT) at RWTH Aachen University. As regarding for the editorial board, we would like to thank all participants contributing to ICBEM & RGC 2018 with their research and hope the conference to be a great experience for all participants.

The Editorial Board:

Prof. Kazuo Yana	Prof. Jaakko Malmivuo	Prof. Steffen Leonhardt

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Proceedings of the 11^{th} International Conference on Bioelectromagnetism 2018, Aachen, Germany, © 2018, International Society for Bioelectromagnetism (ISBEM)

Image processing for semi-automated microscopic analysis of ice re-crystallization process during isothermal annealing

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Introduction

Analysis of complex physical, chemical or biological processes is associated with the analysis of a huge amount of visual material – images. One such process is ice recrystallization, which takes place upon thawing of a frozen fluid-containing sample from the temperature of liquid nitrogen [1]. In particular, the importance of this phenomenon becomes highly significant, when it comes to long-term storage of biological material for medical purposes with the use of low temperatures, so-called cryopreservation process [2, 3]. In turn, microscopy is a widely used method that directly allows obtaining information about the ice crystal being formed, its geometric parameters, and, as a consequence, indirectly study the kinetics of ice (re)crystallization as well as melting process [4]. At the same time, the development of appropriate methods and tools that provide a high-quality automated analysis of microscopic images is a difficult task. The automated analysis of cryomicroscopic images containing living cells has already been performed by our group [5]. Another group has established a sophisticated method to analyze ice recrystallization [6]. However, in this work processing of images is largely based on the Hough transformation, which often leads to failure in image analysis when it comes to more complicated and interconnected structures [7, 8]. Thus, this work is devoted to development of automated methods and tools that provide correct image segmentation and improved analysis of ice crystals and recrystallization process.

Materials and Methods

Cryomicroscopy: The initial data for processing were a series of microscopic images of ice crystals with a size of 1390 x 1038 pixels. An example of the image taken at the beginning of isothermal annealing at -10 °C is shown in fig. 1. The volume of a sample (2 µl) consisting of 0-10% (v/v) dimethyl sulfoxide (DMSO) in common mesenchymal stem cell culture medium (total concentration of fetal bovine serum 20%, v/v) was transferred onto a quartz dish, covered with a cover slip and placed into pre-cooled (4 °C) Linkam cryostage (BCS 196, Linkam, UK). The temperature was controlled with Linkam software (Linksys32, Linkam, UK). The following freezing/thawing protocol was used: cooling rate -10 K/min from 4 °C to -180 °C, equilibration at -180 °C for 5 min, thawing to -10 °C with 10 K/min and further isothermal annealing at -10 °C. The visualization of freezing/thawing was performed using an Axio Imager M1m microscope (Carl Zeiss, Germany). The images were taken with a time lapse of 0.05 s. As an example, fig. 1 shows cryomicroscopic images of ice crystals formed during freezing of solutions without DMSO (0%) and with 2.5% and 10% DMSO as well as their recrystallization upon isothermal annealing at -10 °C.

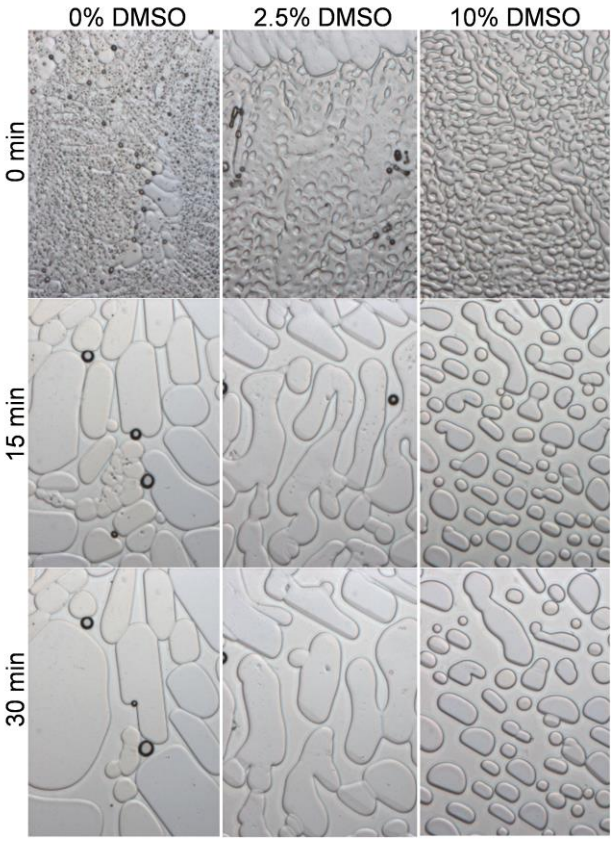

Figure 1: Microscopic images of ice crystals during isothermal annealing at -10 °C showing formation of different ice crystals and their recrystallization. Cryoprotectant used: 10% DMSO

Image processing and analysis: Software development was carried out in the open-source environment Lazarus (license GNU GPL). The first step in image processing is pre-filtering. It should be taken into account that at present most of the used firmware and hardware solutions provide sufficient quality of the used image. Therefore, the need to use this stage is no longer necessary.
The next stage is preliminary segmentation. First of all, one should determine a segmented part of the image. Since the intensity of pixels inside the crystal (I_{in}) can largely coincide with the intensity of the pixels outside the crystal (I_{out}), the use of threshold segmentation to separate the pixels of the corresponding crystals from the

others is not always possible. This can be expressed as follows:

$$I_{in} \approx I_{out} \qquad (1)$$

Therefore, the boundaries of crystals are used as the segmented objects. Here we consider the intensity of the pixels, which is significantly lower than the one of other objects in the image (fig. 1).

Since the images under study may differ depending on imaging conditions, physicochemical properties of cryoprotective solutes used, as well as ice structure itself, the constant value of intensity cannot be used as the segmentation threshold (I_{thr}). Therefore, the Otsu method [9, 10] is applied in our analysis. However, one disadvantage of this method is that this approach does not always allow to accurately determining the required segmentation threshold. In this regard, we considered applying a procedure of setting this parameter by the user. Segmentation of the boundary is carried out using the following expression:

$$\begin{cases} I_o(x,y) = 1; if\,(I_I(x,y) \le I_{thr}); \\ I_o(x,y) = 0; if\,(I_I(x,y) > I_{thr}), \end{cases} \qquad (2)$$

where $I_I(x,y)$, $I_o(x,y)$ are the pixel intensities of the images before and after processing with the coordinates x and y. The result of the processing of the initial image (fig. 2a) is a monochrome image shown in fig. 2b.

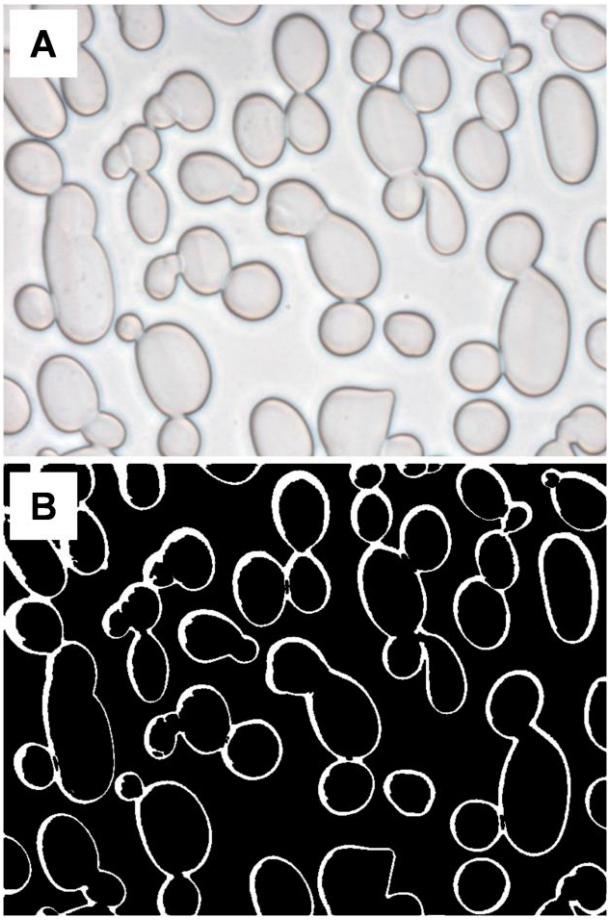

Figure 2: The images of ice crystals before (A) and after thresholding (B). Cryoprotectant used: 10% DMSO

After final thresholding, the resulting image is morphologically processed using erosion and proliferation operations, as described by Perida and coworkers and implemented by our group [11, 12]. This step allows one to remove possible interference with segmentation of the boundary. The resulting segmented sections are grouped together and the contour of each crystal is constructed thereafter.

For each image, a graph of crystal bonds is plotted to show the position of the nearest crystals, due to which growth can occur (fig. 3).

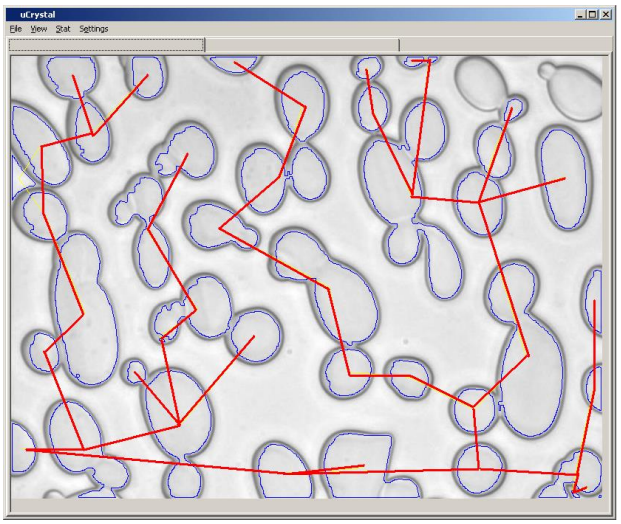

Figure 3: Microscopic image of ice crystals during melting: lines show the graph of the compound between the crystals

Thus, having a series of images, it is possible to calculate a correspondence between the images. This is performed using a distance function, which makes it possible to determine the area of the geometric parameters of the crystals at different stages of the study [12].

Discussion

Cryopreservation is the only way to efficient long-term storage of clinically relevant constructs, such as cell compartments (DNA, RNA), suspended and attached cells, 3D tissue engineered constructs as well as solid organs. It is known that, along with other process parameters, ice recrystallization is one of the main damaging factors during cryopreservation. Thawing of frozen samples in uncontrolled manner often leads to mechanical damage to either cell membrane or tissue leading to impairment of their mechanical properties and thus long-term instability and limited functionality. Analysis of ice recrystallization and a possibility of its inhibition could help to understand main challenges associated with low quality of cryopreserved biological material.

In this work, we considered current information in the field of cryopreservation [13, 14], protection of biological materials from ice formation and its control [3, 15, 16] and analysis of ice recrystallization during isothermal annealing [6]. This yielded significantly improved approach for automated microscopic analysis of ice recrystallization

process. A lack in information on image analysis of ice recrystallization makes discussion of our approach difficult. However, analyzing available information [6] it is possible to suggest that the developed automated approach could significantly shorten a time period required for image processing and analysis. However, there is a number of challenges to be considered. Firstly, it should be taken into account that the automated reconstruction of such formed and melted ice crystals or crystal aggregations becomes even more complicated in case of a complex crystalline structure (fig. 4a).

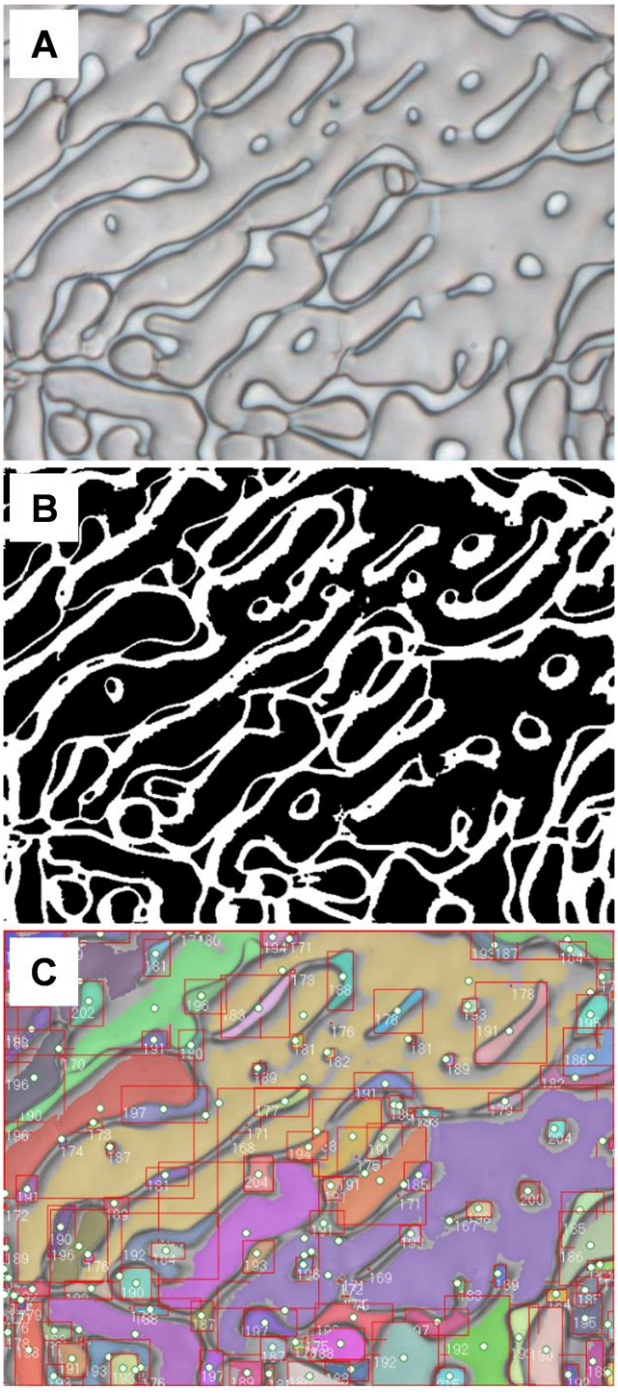

Figure 4: Microscopic images of ice crystals during thawing at -10 °C: initial (A), after thresholding (B) and cluster analysis (C). Cryoprotectant used: 10% DMSO

In this case, fig. 4b shows the result of segmentation and the marked areas of crystals (fig. 4c). As can be seen, free areas are often marked as areas of crystals; the use of internal intensity does not give a complete separation (in fig. 4c, the average intensity (from 0 (black) until 255 (white)) inside the object is known). Therefore, we are currently evaluating a possibility of using form analysis and the boundary of an object's transition to improve image processing. This should allow determining the type of the segmented object. Secondly, ice recrystallization process is monitored within small sample volume (often not more than 5 µl). The initial point for isothermal annealing is determined based on the analysis of melting point using differential scanning calorimeter, which requires 10-20 µl of a test liquid sample. Thus, the difference in volume could lead to mismatched melting points, which often requires decreasing the temperature of isothermal annealing for analysis of ice recrystallization.

Conclusions

Investigation of the process of crystallization is a complex and important task, especially in cryopreservation of biological material for medical purposes. In the current work, the process of processing and analyzing a series of microscopic images during freezing and thawing is considered. Based on available literature as well as our image processing approach, it is now possible to analyze ice recrystallization process during thawing and isothermal annealing. Moreover, using our approach tracking of certain ice crystals can be performed. This knowledge can now be applied for precise determination of the efficiency of inhibition of ice recrystallization of novel and low-toxic cryoprotective agents.

The next stage of research is the improvement of software, taking into account the presence of complex crystalline formations, which cannot be processed using the described approaches. To do this, we should consider the analysis of the shape of the crystal. This could allow us to identify the grouped formations in a different scale as well as areas with little difference in image intensity.

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