Real-Time Label-Free Chemical Visualization of the Cell Cryopreservation Process Using Cryogenic Stimulated Raman Scattering Microscopy

Kuan Luo[1], Taijie Zhan[2,3], Yi Xu[2*], Minbiao Ji[1*]

1. State Key Laboratory of Surface Physics and Department of Physics, Human Phenome Institute, Academy for Engineering and Technology, Fudan University, 200433 Shanghai, China.
2. Institute of Bio-thermal Science and Technology, Shanghai Co-innovation Center for Energy Therapy of Tumors, Shanghai Technical Service Platform for Cryopreservation of Biological Resources, University of Shanghai for Science and Technology, 200093 Shanghai, China.
3. School of Medical and Information Engineering, Gannan Medical University, 341000 Ganzhou, Jiangxi, China.

Email: 23110190050@m.fudan.edu.cn

Abstract

Cryopreservation is widely used for long-term cell preservation, but the dynamic process of cryoprotectant permeation within cellular systems during freezing and rewarming remains difficult to study directly. In this work, we developed a cryogenic stimulated Raman scattering microscopy platform for real-time, label-free, chemically resolved imaging of an cell–water–DMSO mixed system during the cryopreservation process. By selectively mapping different components, the system enables real-time visualization of chemical component distribution under different stages of cryopreservation. Time-resolved imaging further reveals the temporal evolution of DMSO concentration during both cooling and rewarming, providing spatially resolved information on cryoprotectant permeation. These results demonstrate that cryogenic SRS microscopy can serve as a powerful tool for monitoring physicochemical changes during cell cryopreservation.

Method

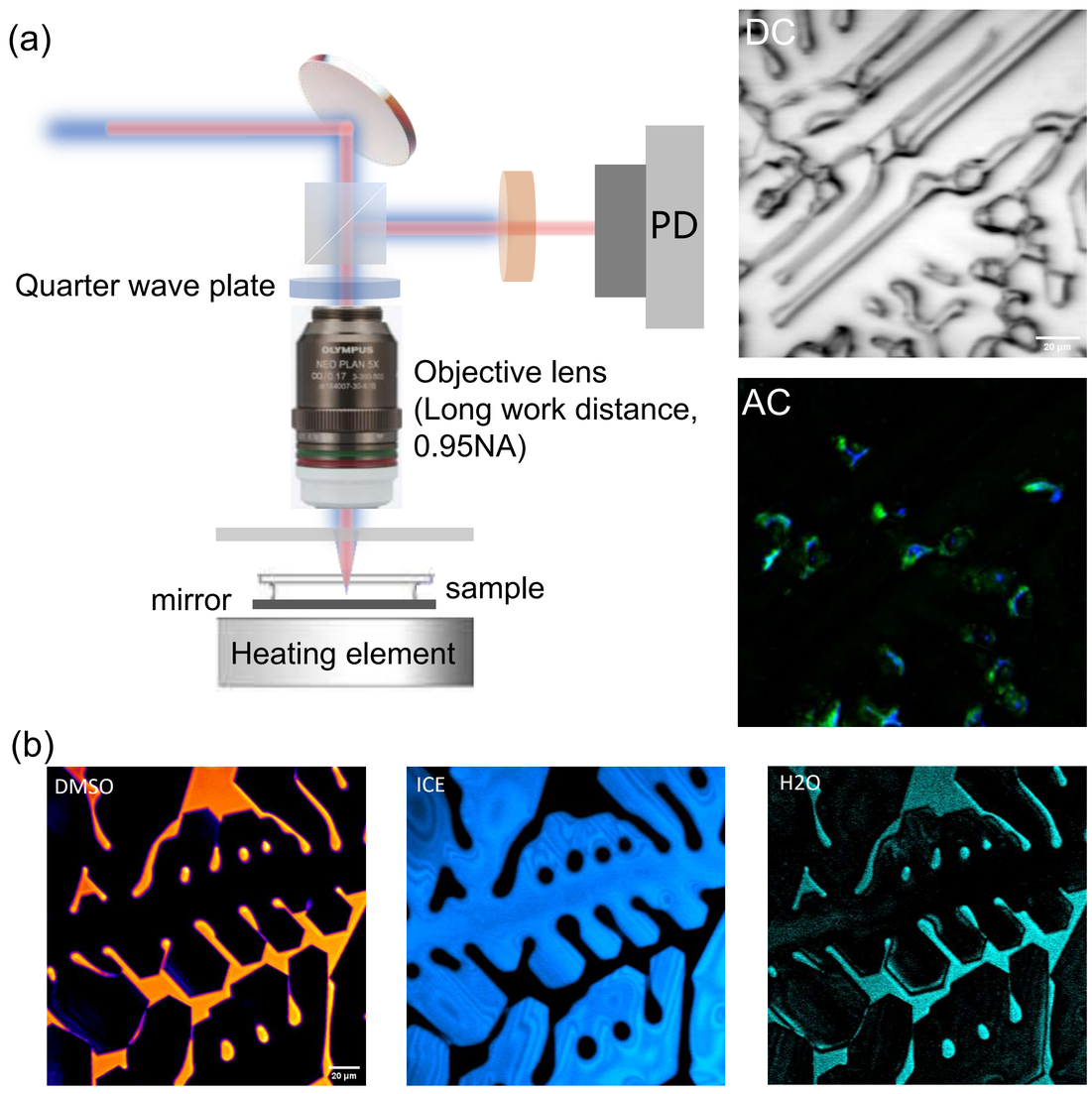

Figure 1. Schematic of of cryogenic stimulated Raman scattering microscopy. (a) Schematic of cryogenic stimulated Raman scattering microscopy. (b) Imaging of different chemical channels in an ice–water–DMSO mixed system.

Result

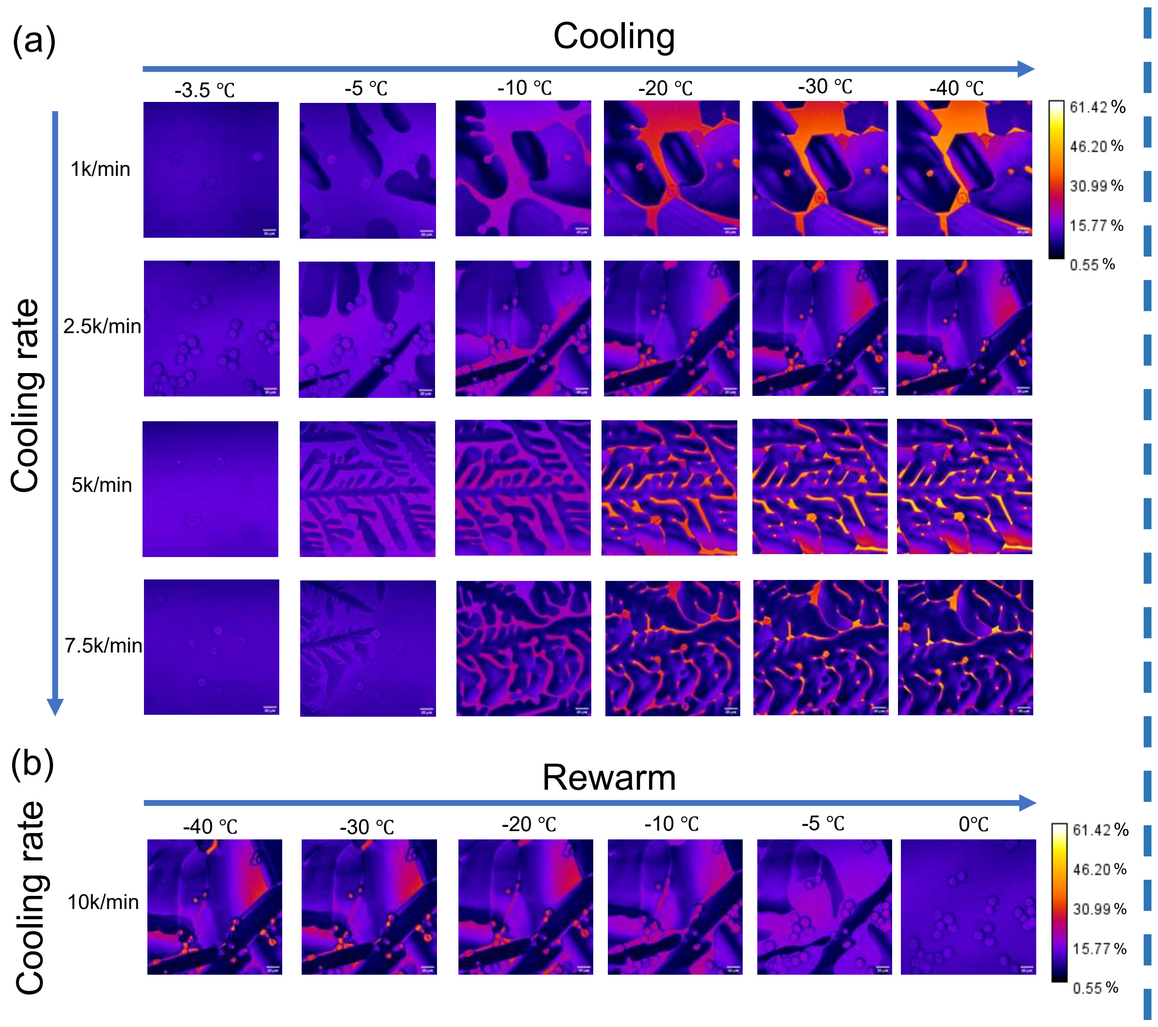

Figure 2. Real-time monitoring of DMSO concentration dynamics during cell cryopreservation. (a) Freezing process under different cooling rates. (b) Rewarming process at a heating rate of 10 K/min.

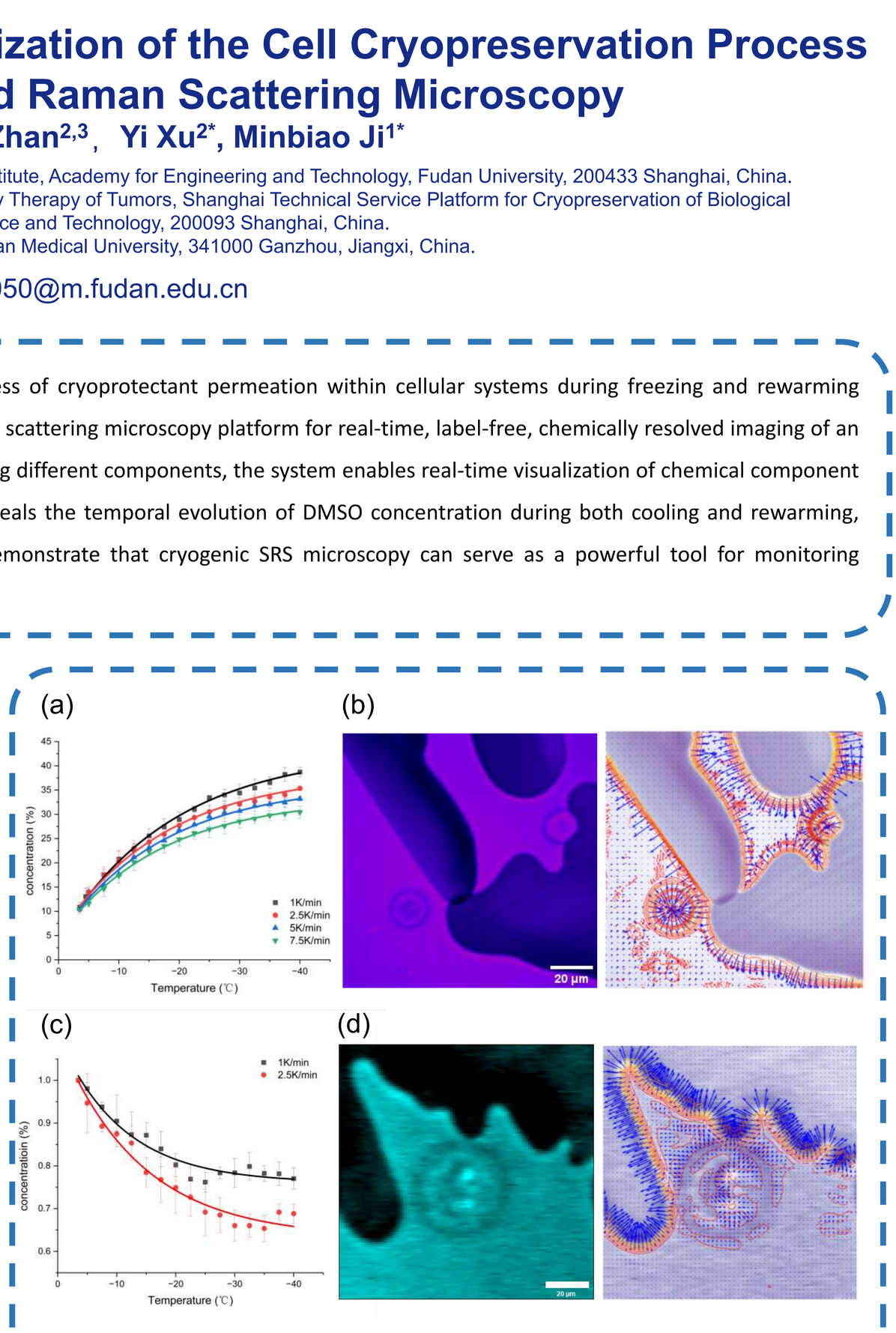

Figure 3. Solution migration process. (a) Intracellular DMSO concentration curves under different cooling rates. (b) DMSO permeation process in the system during cooling. (c) Intracellular H2O concentration curves under different cooling rates. (d) H_2O permeation process in the system during cooling.

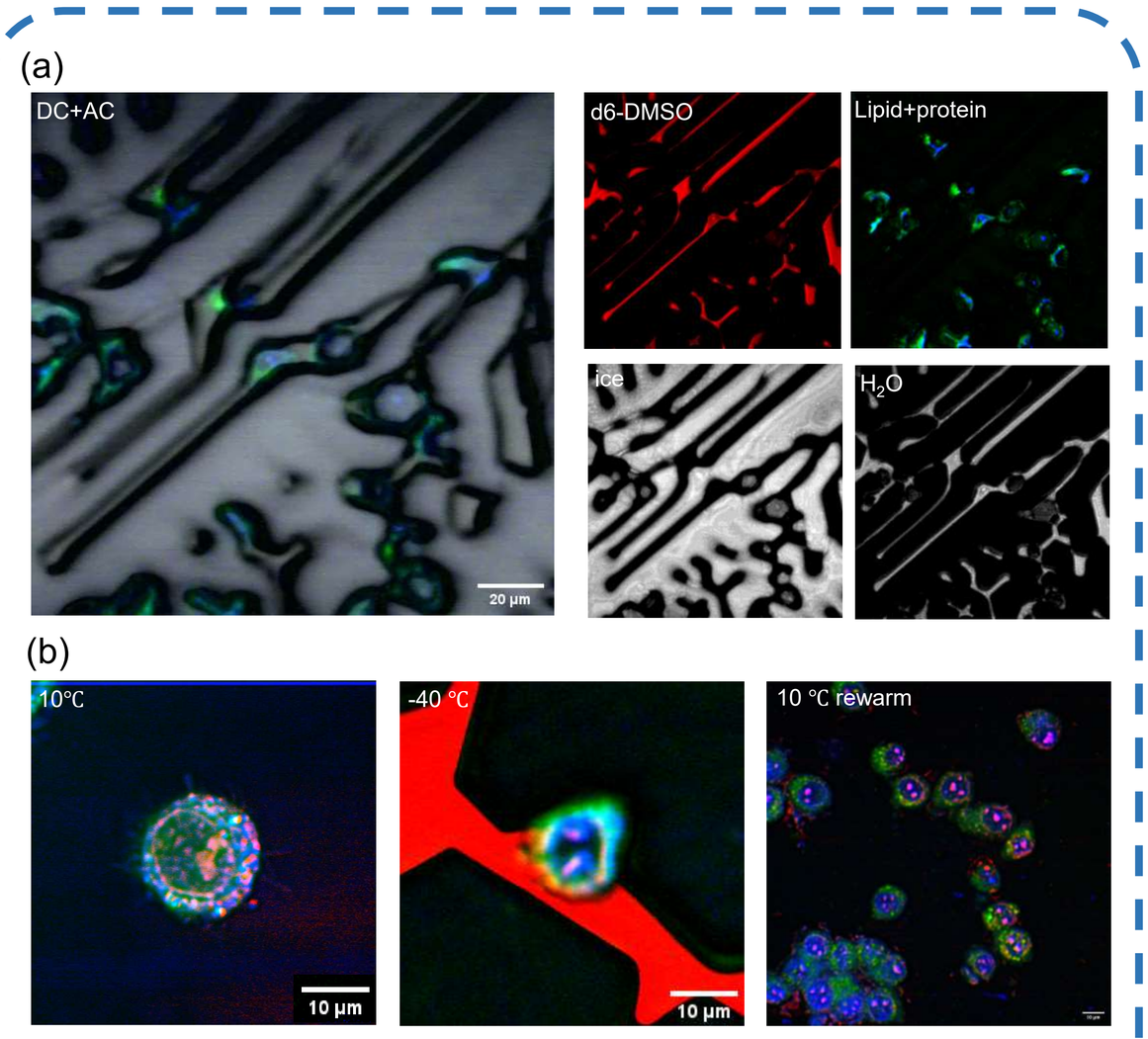

Figure 4. Distribution of different chemical components (a) Spatial distribution of different chemical componentsat −40 °C. (b) Spatial distribution of different chemical components at different stages of cryopreservation.

Conclusion

- Cryogenic SRS microscopy enables real-time, label-free chemical imaging of cell cryopreservation.
- This method allows direct visualization of cryoprotectant permeation in cellular systems.
- Cryoprotectants exhibit a non-uniform intracellular distribution, with local enrichment in specific cellular regions.