Technology Offer

Microfluidic cryo-fixation of biomass, e.g., cells, flagella/cilia, or proteins for (in particular) correlative light-electron microscopy systems

Ref.-No.: 0707-4032-BC

Cryo-Electron Microscopy (Cryo-EM) today is revolutionizing biological research by revealing the structure of cells and proteins with atomic resolution and steadily improving contrast. However, the quality of the results depends critically on the sample preparation. High Pressure Freezing (HPF) and plunge freezing, both pioneered in the 1960s, still represent the state-of-the-art in sample preparation for cryo-EM. Both methods have poor timing precision, disrupt the natural environment of the sample, and preclude continuous light microscopic observation up to the exact time of freezing.

Here, a method was invented to enable the culturing, stimulation, and in-situ cryo-fixation of cells on a microfluidic chip without the need for chemical cryo-protectants.

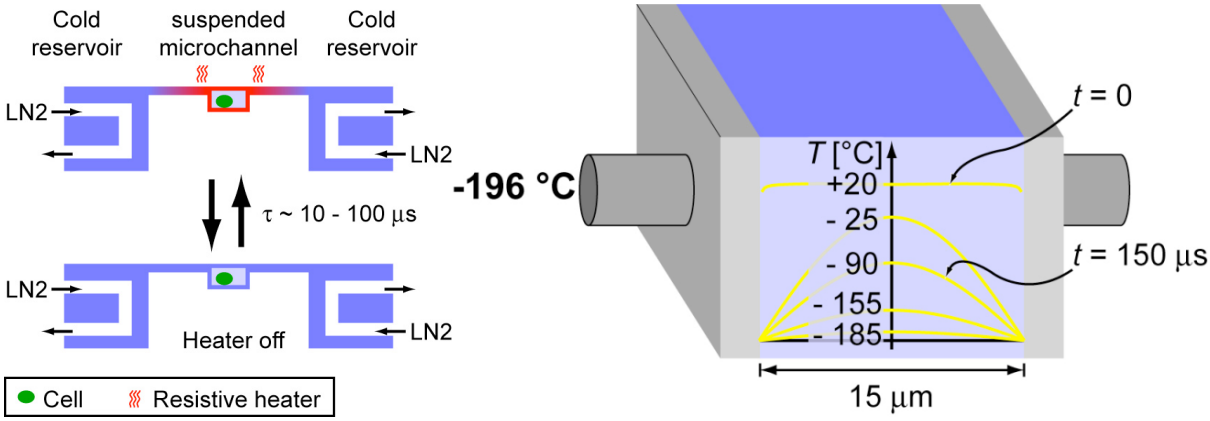

Fig. 1: Schematic of the device. Left: A thin microfluidic channel is attached to a liquid nitrogen (LN2) cooled substrate through heated support beams. When the heaters are turned off, the channel temperature rapidly equilibrates with the substrate. Right: The cooling rate is limited by the thermal conductivity of water. A 15µm wide silicon channel equilibrates fast enough for pure water to vitrify. The center of the volume reaches -90°C in 150µs after the heating is stopped.

The enabling concept is to suspend cells in a freestanding microfluidic channel that has a low volume and a very short thermal time constant. If the channel is connected to a cold reservoir through at least one heated support, cells and nutrients can be delivered while the heater keeps the fluid at room temperature. When the heater is switched off, the channel equilibrates rapidly with the cold reservoir. This results in vitrification of the sample if the thermal time constant is sufficiently short (Fig. 1).

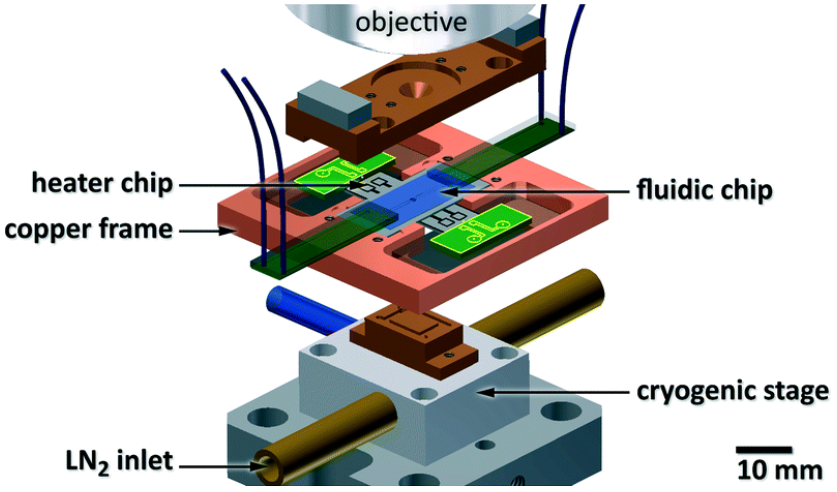

Fig. 2: Assembly of the microfluidic cryofixation device. The channel on the fluidic chip is aligned to the resistive microheater, whose silicon support also serves as a heat sink. A warm copper frame fixes the alignment. Fluid is supplied through two polydimethylsiloxane manifolds with tube connections. Taken from [1]

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Advantages

- Ultra-rapid cooling below critical water crystallization temperature in the rage of 10 to 100µs
- Works at atmospheric pressures
- Uninterrupted visual sample access prior and during freezing

Applications

- Post-fixation optical, Electron, or X-ray microscopy
- Organic single cells as samples (no cryo-protectants needed)
- FIB-SEM systems (cf. [2,3])

Background

Cryofixation yields outstanding ultrastructural preservation of cells for electron microscopy, but current methods disrupt live cell imaging. Here a microfluidic approach is demonstrated that enables cryofixation to be performed directly in the light microscope with millisecond time resolution and at atmospheric pressure without using chemical cryo-protectants. This provides a link between imaging/stimulation of live cells and post-fixation optical, electron, or X-ray microscopy.

Technology

Microfluidic technology provides an excellent platform for applications that require localized heating and cooling with short time constants. Some examples are briefly outlined within the electronic supporting information of Ref. [1]. Compared with these studies, the work described here overcomes three key challenges. The first is to approach initial cooling rates of at least ~10^4 °C s^{-1} to suppress ice crystallization. Secondly, the final temperature must be below −140 °C to prevent de-vitrification; and third, continuous flow of fresh media is required during live imaging prior to freezing. These points are addressed in the design by

1) minimizing the thermal mass of the channel containing the sample,

2) minimizing the distance between the cold surface and the sample, and

3) maintaining uninterrupted, room-temperature connections into and out of the heated microchannel.

In particular, it was found that microfluidic perfusion devices of extremely low thermal mass could be made using microfluidic polymer foils in combination with silicon micromachined components.

Publications

[1] **Mejia**, Y. X., Feindt, H., Zhang, D., Steltenkamp, S., & Burg, T. P. (2014). Microfluidic cryofixation for correlative microscopy. *Lab on a Chip*, *14*(17), 3281–3284. https://doi.org/10.1039/C4LC00333K
[2] **Fuest**, M., Nocera, G. M., Modena, M. M., Riedel, D., Mejia, Y. X., Burg, T. P. (2018). Cryofixation during live-imaging enables millisecond time-correlated light and electron microscopy. *Journal of Microscopy*, 272 (2), 87-95. https://doi.org/10.1111/jmi.12747
[3] **Fuest**, M., Schaffer, M., Nocera, G. M., Galilea-Kleinsteuber, R. I., Messling, J.-E., Heymann, M., Plitzko, J. M., Burg, T. P. (2019). In situ Microfluidic Cryofixation for Cryo Focused Ion Beam Milling and Cryo Electron Tomography. *Scientific Reports*, 9, 19133. https://doi.org/10.1038/s41598-019-55413-2

Patent Information

[4] PCT (*WO2009065585A3*), EP, US, JP

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