Technology Offer

Cryo-Light Microscope and Immersion Medium for Cryo-Light Microscopy

Ref.-No.: 0707-5496-BC

Cryogenic fluorescent light microscopy of flash-frozen cells presents significant advantages and provides an important complement to electron cryo-microscopy. In particular, bleaching decreases drastically at low temperature while the fluorescence yield of many fluorophores increases and the spectral bands narrow. The application of modern super-resolution methods such as STED, PALM, STORM, or SIM at cryogenic temperature holds the prospect of imaging fluorescent proteins with high precision in 3D and correlating their localization with the ultrastructure seen in electron cryo-microscopy of the same sample. To attain the highest level of resolution, aberration-free immersion objectives with accurately matched immersion media are required, but both do not exist for imaging below the glass transition temperature of water.

Only an immersion medium suitable for temperatures below -135 °C will allow for imaging a high pressure frozen water containing biological samples without altering the structure of the frozen water or the sample therein. Above this temperature, the amorphous water in the sample will convert into crystalline water. This conversion will affect the sample due to the increased volume of crystalline water and due to alterations in solubility of biological molecules in crystalline and amorphous states of solid water; it will also change the optical properties of the sample.

Technology

The present technology is a new approach towards immersion light microscopy at -140 °C. A new immersion medium, HFE- 7200, which matches the refractive index of room-temperature water at cryogenic temperature is combined with a concept in which the objective is not in thermal equilibrium. The temperature drop is maintained by actively heating the ceramic lens mount along the perimeter near its connection with the objective body (s. Fig. 1). The ceramic is chosen by its low thermal conductivity and low thermal expansion coefficient creating a thermally shielded microenvironment around the sample. Thus, refractive index gradients due to temperature variations in the liquid are minimized and not likely to distort the wavefront.

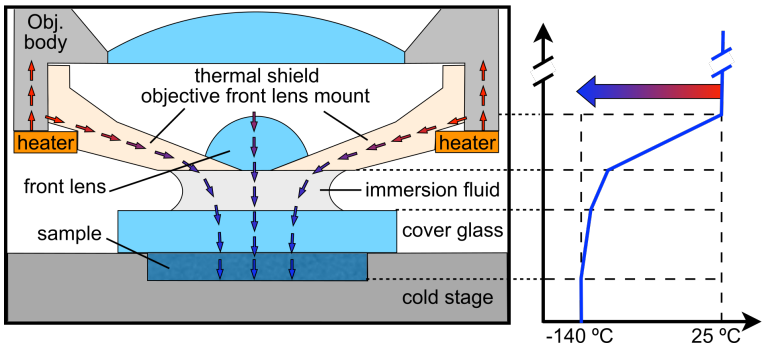

Figure 1: Set-up of the objective for cryo-light microscopy and temperature profile therein.

Nevertheless, there is a heat flow from the front lens holder through the immersion medium towards a sample to be imaged. Thus, the immersion medium, particularly when using a glass cover on the sample, is slightly warmer than the sample. As a result, the sample may even be kept at or slightly below the freezing point of the immersion medium without freezing the liquid immersion medium.

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A suitable immersion medium was found in partially fluorinated liquid ethoxynonafluoro- butane (HFE-7200), having a surprisingly low refractive index of 1.28 at room temperature and a liquid range from >70 °C to below -140 °C. HFE-7200 is also inexpensive, non-toxic, and safe for the environment. However, various other hydrofluoroethers and their mixtures can be used as well.

One of the key advantages of immersion objectives over air objectives is their light collection efficiency, which grows as ~ NA^2. Indeed the inventors measured an increase in brightness of 5.7±0.6 times from a 63×/0.75 air objective to the 63×/1.15 immersion objective accord- ing to the present invention at -140 °C. This is in agreement with the expected scale factor of ~ NA^4 for wide-field fluorescence imaging.

It could be shown that the present invention can provide superior contrast in yeast cells expressing fluorescent proteins. A considerable improvement of both signal-to-noise ratio and image quality is achieved in cryo-conditions where the photobleaching is suppressed around 64 times (s. Fig. 2). In addition to it, in immunostained human bone osteosarcoma epithelial cells (U2OS) networks of mitochondria and vimentin filaments are well resolved simultaneously (s. Fig. 2C).

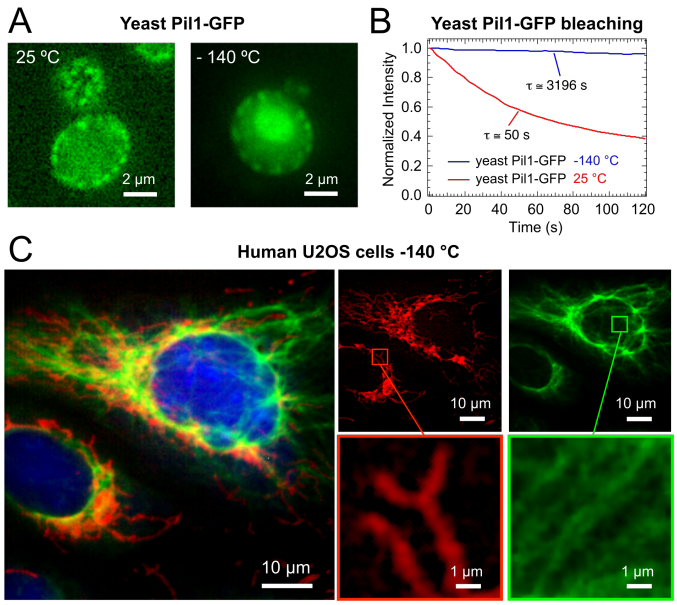

Figure 2: (a) room temperature (left) and cryogenic (right) wide-field fluorescence images of yeast cells ex- pressing GFP tagged Pil1; (b) photobleaching curves for GFP at room temperature (red) and at -140 °C (blue), in cryo-condition the GFP bleaching is suppressed about 64 times; (c) threecolour wide-field cryo-fluorescence image of plunge frozen U2OS cells labelled with Alexa Fluor 488 (vimentin cytoskeleton), Alexa Fluor 594 (Tom20 mitochondrial protein), and DAPI (cell nuclei).

Advantages

- Decreased bleaching and increased and narrowed fluorescence.
- Immersion objective increases light collection efficiency.
- Reduced heat flow from microscope to sample.
- No need for equilibrating microscope.
- No vacuum required for contamination-free long-term imaging.

Patent Information

- EP patent application filed in September 2017.
- US patent application pending.

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