Improved Frozen Specimen Imaging for X-ray Cryomicroscopy

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ABSTRACT

In the presence of water vapor, frost will develop and rapidly accumulate on the surface of frozen, hydrated biological samples over time. As the amount of frost increases on the sample, it will obscure an observer’s ability to accurately collect data and properly resolve key features at the micron and submicron levels. Conventional mechanisms to combat frost formation involving active systems, like vacuum or nitrogen gas purge, have noted limitations that compromise their effectiveness. Gas purging risks convective sample heating and vacuum traditionally requires high vacuum pressures (10−7 – 10−4 Torr). In addition, both systems are required to be in continuous operation during sample evaluation.

We developed a concentric frame of ice along the periphery of a biological tissue sample mounted on a silicon nitride membrane. The sample was held at cryogenic temperatures well below the glass transition temperature for ice (T ≤ 135 K). The ice frame acts as a hygroscopic surface and passively attracts water vapor away from the surrounding environment and to its own surface, via preferential nucleation. Using image processing techniques, we were able to quantify the ice frame’s ability to reduce the total amount of frost accumulation on the silicon nitride membrane over a three-hour evaluation. With the addition of the ice frame, the overall window clarity approximately doubles over time going from 36% to 60% after three hours. The ice frame demonstrated considerable ability as a mechanism to manage frost on frozen biological samples.

Keywords: Silicon Nitride; Hygroscopic; Frost; Image Processing

INTRODUCTION

Water Vapor Nucleating and Freezing on Window Surface

- Frozen, hydrated biological specimens are supported by silicon nitride (Si3N4) windows for X-ray fluorescence microscopy
- The silicon nitride window is held within a cryostage at cryogenic temperatures (T ≤ 135 K)
- The sample chamber is continuously inducted with nitrogen gas to purge water vapor from its environment
- Residual water within the sample chamber accumulated on the window surface, gradually obscuring the transparency of the silicon nitride membrane

Evaluating Frost Accretion on Sample Window

RESULTS

Visual Observations

<table>
<thead>
<tr>
<th>Purge, Minus Ice Frame</th>
<th>LN2 Temp, 0hr</th>
<th>LN2 Temp, 1hr</th>
<th>LN2 Temp, 2hr</th>
<th>LN2 Temp, 3hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purge, Plus Ice Frame</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No Purge, Plus Ice Frame</td>
<td></td>
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</tbody>
</table>

Graph 1: The average luminance of the RED fluorescent tags attached to the blood vessels over time

Graph 2: The average luminance of the GREEN fluorescent tags attached to the amyloid over time

The average relative luminance was highest for cases where the ice frame was present.

CONCLUSION

1. Preferential nucleation on the ice frames surface reduces the frost on the sample. The implementation of an ice frame presents an alternative mechanism to remove frost from frozen, biological samples undergoing X-ray cryomicroscopy and can indirectly make the sample colder
2. The ice frame outperformed a conventional active mechanism, nitrogen purge, in removing water vapor from the environment
3. Establishing ice frames as a part of sample preparation workflow for X-ray cryomicroscopy experiments allows cold temperature savings, due to the removal of convective heat fluxes from nitrogen gas. This may allow frozen samples within the cryostage to be exposed higher X-ray photon fluxes without the concern of photo-oxidation

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