Thermoelectric Freezer for Reducing Radiation Damage to Biological Samples in Synchrotron Microprobe Experiments

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Introduction

Synchrotron x-ray microprobes are frequently used to study the chemistry of microscopically inhomogeneous natural systems, such as plant roots, bacteria, and soils. Experiments on these systems in their hydrated state face a serious challenge from radiation damage. Beam-induced dissociation of water molecules dramatically alters local chemistry, and cell tissues quickly disintegrate in the focused x-ray beam. Freezing has been shown to significantly slow radiation damage in synchrotron experiments on protein crystals, but while LN_2 or LHe cryojets are now a standard feature of most protein crystallography beamlines, the freezer geometry of the cryojet is not well-suited to the wide variety of sample shapes and sizes brought to a microprobe user facility.

The requirements for freezing samples in the microprobe include these: the freezer must fit into the limited space defined by the focal lengths of the microprobe optics and detectors; the sample must not vibrate and be able to be reproducibly positioned to ±0.1 µm; the freezer geometry must minimize elastic scattering and fluorescence background into the detectors; the transmitted beam must be able to be measured; the sample must be visible for optical microscopy; and the sample and windows must not develop frost over the course of the user run, typically 2 to 5 days. Additional desirable features include easy set-up and operation; the ability to handle a wide range of sample sizes and shapes; rapid sample exchange; need for only minimal intervention during an extended beam run; and low cost to build and operate.

Methods and Materials

We have built a small, lightweight freezer based on an annular thermoelectric cooler (TEC) [1]. The annulus allows transmission through the sample and minimizes elastic scattering into the solid-state detector used for fluorescence measurements. Sample temperatures of –40°C have been achieved within 5 min of power-up from room temperature by using a single TEC with a water-cooled heat sink. The total cost, including shop time, TEC, and connectors, for three cooling stages with different apertures and cooling powers was under $3,000. We use a general-purpose power supply that is shared with other experiments and a closed-cycle recirculating chiller with water at 5°C for the heat sink. We observed no vibration of the stage due to the water flow. Some light frost on the outer Kapton® window occurred after 3 days of continuous use in the sultry Illinois summer. This was easily removed by letting a slow stream of dry He flow across the window face.

The face of the stage is angled at 30° to the incident beam direction for the PNC-CAT microprobe station at the APS. The minimum sample-to-beam distance in this configuration is 35 mm, which could be made smaller by trimming the long side of the Al wedge of the cold plate. The total weight is 1200 g. Overall size is 100 × 100 × 85 mm, including three layers of styrofoam insulation to prevent condensation. A double Kapton window in front of sample provides additional thermal insulation, but the sample is still visible to the two microscope cameras. The sample cells hold wet samples between two thin sheets of Kapton foil and can be pre-frozen so they can be quickly swapped in and out of the freezer.

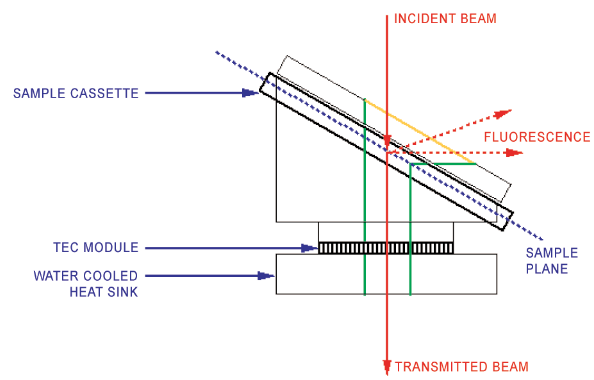

FIG. 1. Schematic drawing of TEC freezer for synchrotron microprobe fluorescence measurements.

Results

The TEC freezers have been used for microprobe fluorescence maps of *Acetabularia acetabulum* collected at beamline station 20-ID-B on the microprobe and for extended x-ray absorption fine structure (EXAFS) and x-ray absorption near-edge structure (XANES) experiments on beamline stations 13-ID-D, 13-BM-D, and 20-BM-B.

Discussion

The TEC-based freezer presented here is inexpensive, easy to set up and operate, and adequate for reducing radiation damage in a wide range of synchrotron experiments. The modular sample cassette is sufficiently flexible to accommodate the needs of most users. These freezers are in regular use at PNC-CAT and GSECARS. The entire freezer, with power supply and chiller, is portable and available for loan to other APS beamlines. Planned improvements to the design include the use of stacked TEC modules to achieve lower temperatures.

Acknowledgments

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Reference

[1] Melcor, *TEC design guide*, available on web site, http://www.melcor.com (2003).