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Monitoring the Temperature of a Cryogenic Stage for Cryo-EM

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We have been developing a software system, called Leginon, for the control and acquisition of images from a transmission electron microscope [1-3]. This software allows for the automatic acquisition of large numbers of images under low dose conditions from a specimen embedded in vitreous ice. One obstacle in developing a fully automated system has been the need to monitor and replenish the cryogens used to maintain the temperature of the vitreous ice specimens. We use a Gatan cryo-specimen holder (see figure 1) that uses a liquid nitrogen filled dewar to cool the specimen. Heat is transferred from the specimen tip by means of a metal bar that is connected to the tip and runs through the center of the dewar. When the dewar is full, the bar is completely immersed in liquid nitrogen but, as the level of cryogen in the dewar drops due to boiling the bar eventually is immersed only in liquid nitrogen vapor. Our observations during extended experiments suggest that this change in level of cryogen leads to small temperature changes that cause the specimen to drift. We also note an extended period of drifting when the dewar is refilled, which is currently done approximately every two hours. Waiting for the drift to reach acceptable levels is currently the most important factor limiting high throughput data acquisition.

We have developed a prototype of a system for monitoring the temperature of the cryostage accurately enough to be able to determine the level of liquid nitrogen within the dewar. The device we have developed (schematically illustrated in figure 2) takes advantage of the existing temperature sensor in the tip of the Gatan cryostage holder. This sensor is made of a silicon diode, which when subjected to a constant current, delivers a voltage proportional to the temperature. Our device sends a constant current of 10μA into the cryogenic stage and the output voltage is connected to a precision multimeter. These small voltage changes were systematically monitored over an extended time period during which the level of cryogen in the dewar was determined by inspection and periodically replenished. The results are shown in figure 3. As the figure indicates, a voltage change of about 500 μV is associated with the change in cryogen level as the dewar empties. This voltage change corresponds to a change in temperature of approximately ~0.25 K. While this temperature change is very small, it appears to be reproducible as shown by the consistent periodicity of the behavior between the fill cycles.

Future developments will concentrate on thoroughly characterizing a variety of cryostages to determine whether the observed temperature of the specimen tip can be used as a generally reliable means of determining the level of liquid nitrogen in the dewar. We will also correlate these measurements with observed measurements of specimen drift over extended data collection cycles. Ultimately we hope to develop an automated system for replenishing the liquid nitrogen in the dewar that will maintain the cryogen at a level which leads to the most stable conditions for data collection.

References
1. Potter, C.S., et al. (1999) Ultramicroscopy, 77, 153-161.
2. Pulokas, J., et al. (1999) JSB. 128, 250-256.
3. Carragher et al. (2000) JSB. 132, 33-45.
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Figure 1: Gatan cryostage specimen holder.

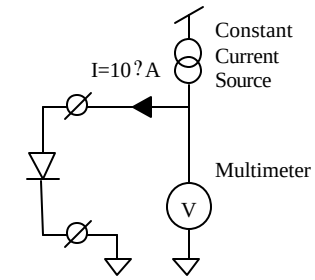

Figure 2: Schematic diagram of temperature sensing device.

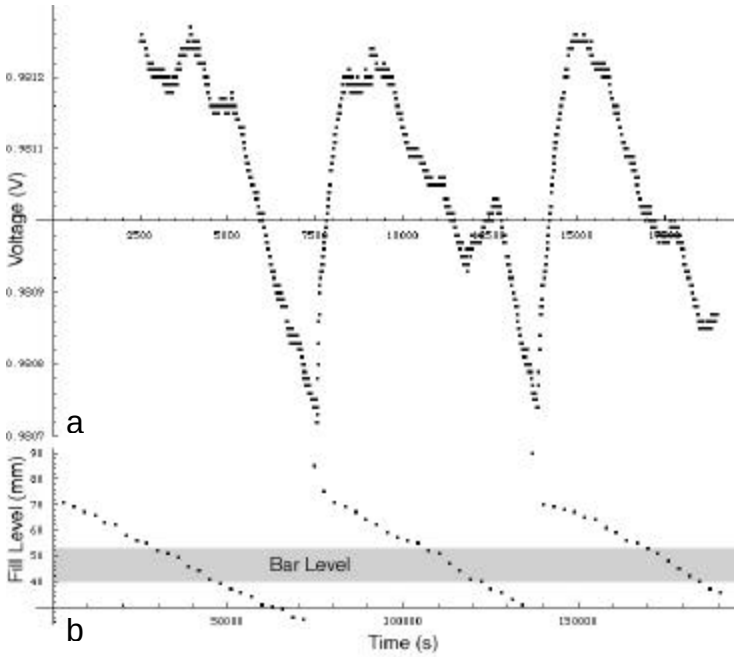

Figure 3: a) Temperature in the dewar is measured by monitoring voltage as described in the text. b) the level of cryogen is simultaneously measured by inspection.

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