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HHMI Tecnai F30 Helium Microscope: Initial Results and Observations

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The incentive for viewing biological samples at liquid helium temperatures comes from reports of a decrease in dose sensitivity at these temperatures [1]. This could be exploited to obtain images with higher signal-to-noise ratio, which for single-particle specimens translates into higher alignment accuracy and the potential for higher resolution. On the other hand, the density of amorphousice increases to 1.06 g/cm^3 from 0.94 g/cm^3 below 38 Kelvin [2]. A corresponding change in electron scattering was observed [3]. This raises the possibility that the resulting decrease in contrast for biological specimens might offset or even outweigh the potential benefit gained by dose protection. For single particle reconstruction projects this would mean that it would be harder to find and align individual molecules.

The Tecnai F30 Helium instrument provides the unique opportunity to examine thesame specimen under identical conditions at both liquid nitrogen (84 Kelvin) and liquid helium (11.6 Kelvin) temperatures. (A pleasant byproduct of the engineering necessary to achieve this capability is an extremely stable cryo stage. The stage is able to maintain 84 Kelvin for approximately 28 hours and 11.5 Kelvin for approximately 7 hours.)

The aim of this study is to measure the contrast of some typical single-particle specimens at both temperatures. In an initial experiment with *E. coli* ribosomes, grids were prepared for cryo-EM in a standard way [4]. Images were taken at both temperatures at various defocus settings. 4712 particles were classified into 83 views by 3D projection alignment with an existing 3D reference [5]. Particles falling into a particular view were averaged. Between 28 and 43 particles contributed to each of these averages (Fig. 1). The contrast value of the averages was derived by integrating the density values within the particle and comparing it to the integrated density outside the particle.

For the ribosome specimen, there is no evident change in contrast measured in this way (Fig.2). Both curves (He and N) have an expected upward trend as a function of defocus, but the fluctuating behavior is probably due to statistical error. We intend to repeat this experiment with a specimen that consists of protein only (e.g., calcium release channel; [6]) as the putative contrast change would be maximal for such a specimen.[7]

[1] W. Chiu et al., J. Microsc.,141 (1986) 385-391.
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[6] M. Radermacher et al., J. Cell Biol., 127 (1994) 411-423.

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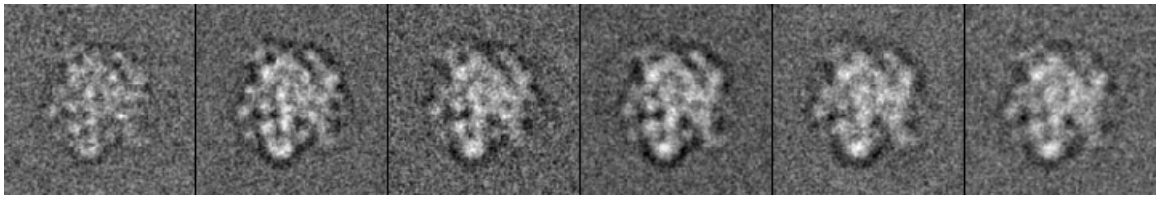

FIGURE 1A Averages of Ribosome at 11.4 Degrees Kelvin

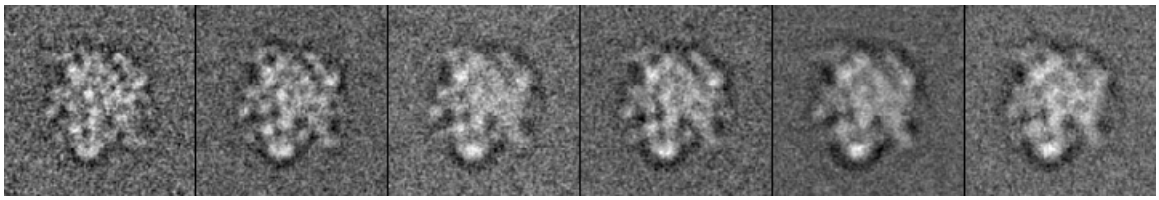

FIGURE 1B Averages of Ribosome at 84 Degrees Kelvin

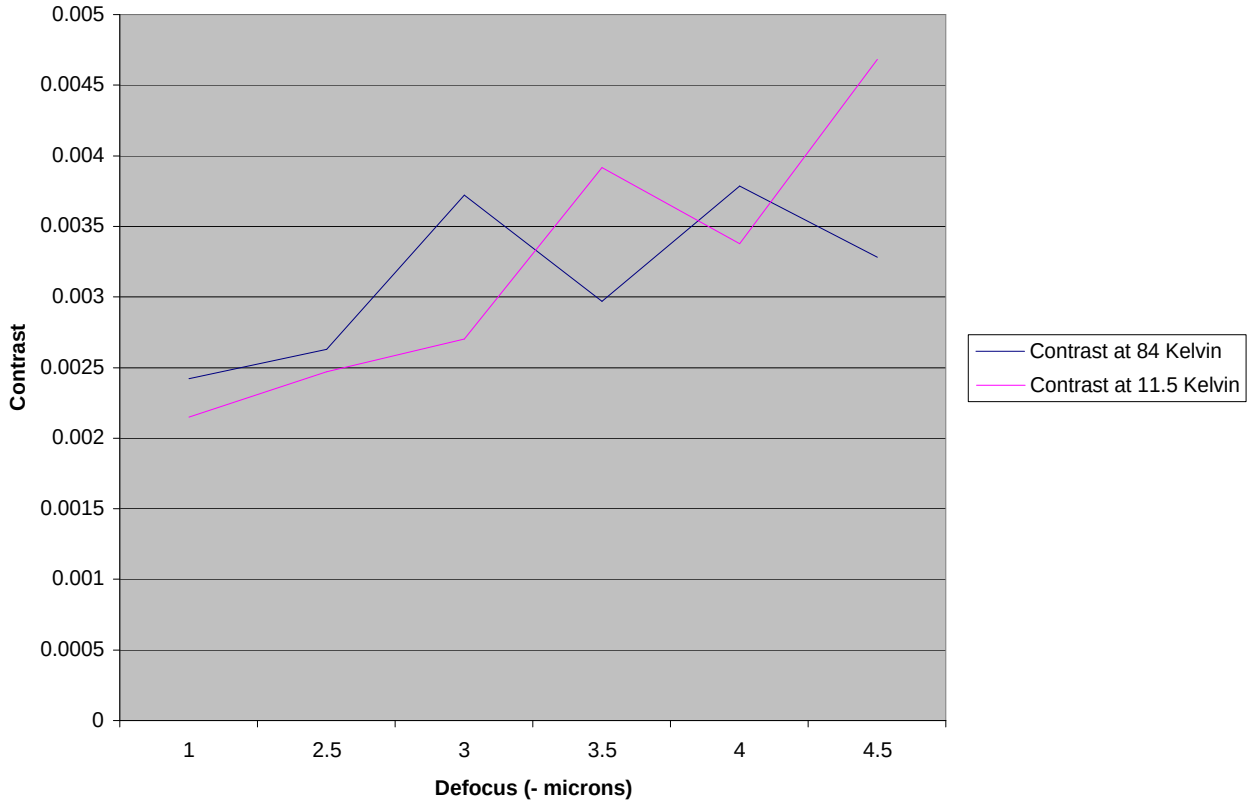

FIGURE 2. Graph of Contrast Vs Defocus at 84 Degrees Kelvin and 11.5 Degrees Kelvin

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