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IT-10-P-2098 Cryo-STEM Tomography for 3D Analysis of Cell Structure

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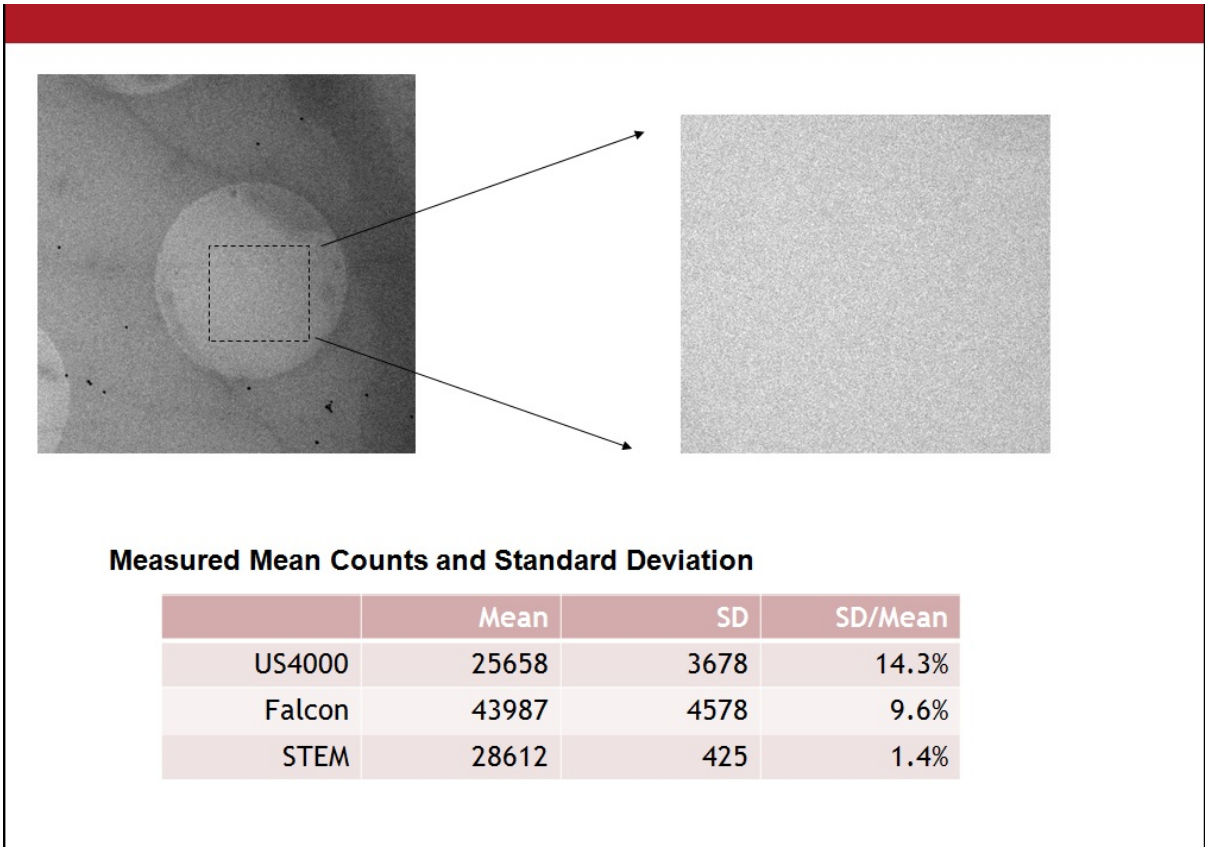
Recently, several studies for observation of biological specimens as plastic section have been performed by using STEM, and the potential has been indicated. STEM tomography offers several important advantages including: (1) it is effective even for thick specimens, (2) 'dynamic focusing', (3) ease of using an annular dark field (ADF) mode and (4) linear contrasts. It has become evident that STEM tomography offers significant advantages for the observation of thick plastic specimens. In this study, the technique applied for Cryo-specimens. Of course, even in Cryo-Tomography, the advantages of STEM above mentioned are valid. Because STEM has advantage to resist specimen thickness, it is expected to be powerful method for observing whole cell structure in Cryo-microscopy without thin sectioning.

The insufficient contrast is one of the serious problems in Cryo-electron microscopy. Therefore, the image contrasts by TEM and STEM have to be compared carefully and quantitatively. Figure 1 shows the comparison of the image qualities. The specimen was vitreous ice on Quantifoil made by standard procedure of Vitrobot. Titan Krios equipped with 2 cameras and STEM system was used for the experiment. TEM images were taken by CCD camera (Gatan US4000) and direct detected CMOS camera (FEI Falcon). STEM image was taken in bright field mode. The imaging conditions, image pixel size and the number of irradiation electrons, were normalized. The average counts of the pixels and the standard deviation (SD) were measured for each image, and then SD/mean was calculated. The result was clear that the STEM image had very low back ground noise. This character can be explained theoretically, there are several seasons; (1) Short operation time for pixel (dual time vs. exposure time), (2) Large physical size of the detector, (3) Very small collection angle (same as very small objective aperture in TEM imaging).

By applying Cryo-STEM tomography, clear membrane structure of organelle appeared without staining and without sectioning.



Measured Mean Counts and Standard Deviation

	Mean	SD	SD/Mean
US4000	25658	3678	14.3%
Falcon	43987	4578	9.6%
STEM	28612	425	1.4%

Fig. 1: A comparison of the image quality in Cryo-EM