

# Environmental and In Situ SEM/TEM

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### Preventing charging of liquid cell silicon nitride windows for phase contrast *in situ* TEM

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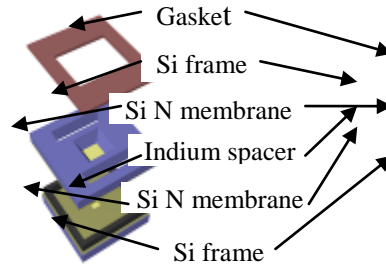
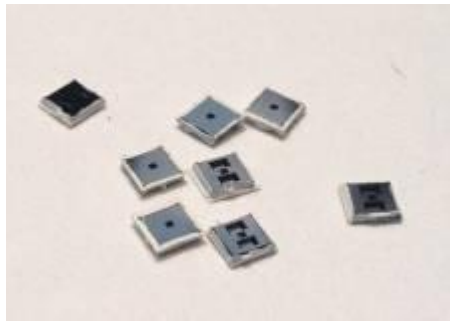
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Liquid cells with thin silicon nitride (SiN) membrane windows (Figure 1) that enclose liquid samples are essential for *in-situ* transmission electron microscope (TEM) both for material and life sciences. [1-4]. However, for biological samples the image contrast is substantially reduced due to the low difference of the density between specimen and liquid. Image contrast can be improved by Zernike-type carbon phase plates inserted in back focal plane of TEM [5] and by image capture with fast and sensitive direct electron detection C-MOS camera [6]. This combination allows capture of dynamic processes with high contrast and relatively low doses.

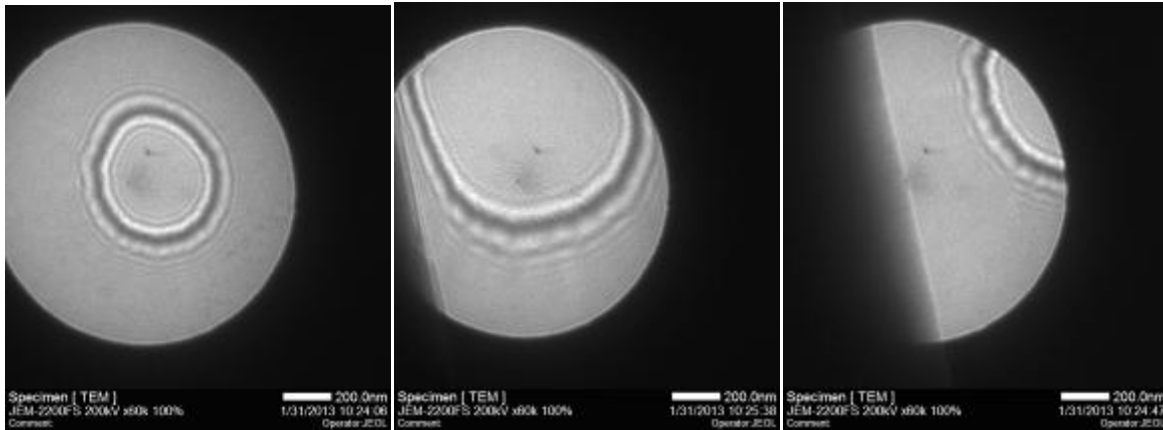
A current drawback of the liquid cells charging of the windows when exposed to electron beam [2] which leads to unintentional beam focusing as shown in Figure 2.

We demonstrate that distortion of the beam focusing is eliminated by carbon coating of SiN membranes in liquid cells as illustrated in Figure 3. By solving the charging problem, we can demonstrate the possibility of imaging biological samples at room temperature and in liquid water by phase contrast in our liquid cell.

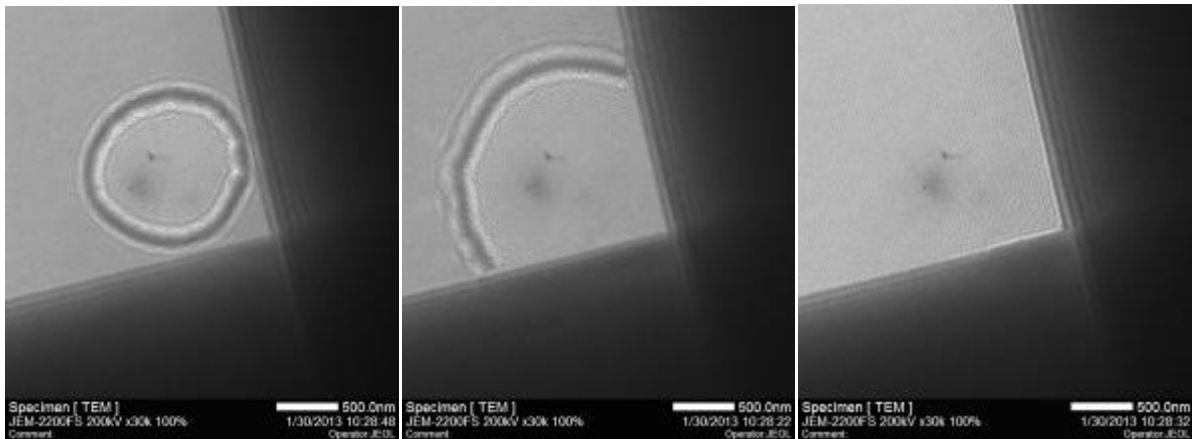
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**Figure 1.** Photo and schematics of liquid cell. The size of the liquid cell is 2.8 mm squared. The window size is 3 by 50 micrometers.



**Figure 2.** Images of deformed central holes of the phase plate. The window of the liquid cell is not isotropic shape with high aspect ratio, the potential is much varied according locations. Thus the appeared hole size and position of the hole were always changed and moving especially near the edge of the window. Left: image of an anisotropic and focused shape of the phase plate's central hole. Center and right: images of an anisotropic shape of the phase plate central hole near the edge of the window, which is deformed and enlarged by the edge of the liquid cell (edge effect).



**Figure 3.** Images of un-deformed central hole of phase plate after carbon coating. Left and center: Hole shape is isotropic and is not deformed or enlarged by the edge effect even at the corner of two edges of the liquid cell. We can enlarge the hole size using the brightness dial as above images. Right: Finally, we can make an ideal condition of parallel beam and achieve effective phase contrast.