Prevention of heavy charging of silicon nitride membrane of liquid cells with phase contrast observation

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Liquid cells with thin silicon nitride membrane windows that enclose liquid samples are essential for *in-situ* transmission electron microscope (TEM) both for material and life sciences. Our group have developed the liquid cells [(1-3)] and try to observe both materials and biological samples in liquid [(4)].

In order to improve image quality (contrast and time resolution) samples in liquid cell, we planned to install JEOL JEM-2200FS with two advanced technologies; phase contrast with Zernike-type phase plate system made by thin carbon film on the back focal plane of TEM [(5)] and fast direct detection C-MOS camera [(6)]. Phase contrast TEM enhancement of contrast especially for low contrast bio specimen, which composed of low atomic number atoms. C-MOS camera can improve the time resolution of the images more than video rate, which prevent the moving of specimen in liquid. This combination allows capture of dynamic processes with high contrast. Moreover both technologies are good for biological samples because we can reduce total dose, which directly related to the radiation damage of the weak biological samples.

Our main target sample of observation with the new microscope is wet biological samples in liquid cell, but there was serious charging problem of the silicon nitride based liquid cells. The silicon nitride membrane windows were heavily charged induced by electron beam of TEM. I tested of using the liquid cells on JEOL 2200FS microscope, but we could not use. The electron beam could not be focused properly due to the heavy charging of the membrane windows. Especially with phase plate system, the charging problem is very sensitive and we cannot use the system unless we solve the charging problem.

I tried solving the problem, and I have already found the solution using carbon deposition on the surface of the membrane by just few nm thicknesses. I am now writing the paper summarizing the result not qualitatively but quantitatively. I have done several additional measurement of surface potential of the membrane using Kelvin probe force microscope (KPFM), a type of atomic force microscope (AFM) mode. Reduction of charging by carbon coating can be explained as quantitative surface potential discussion as shown in Figure 1. Non-carbon-coated membrane windows were heavily charged and these surface potential was around 400 mV after irradiation of electron beam. In contrast, the surface potential of carbon coated (thickness: 5 nm) membrane windows were reduced into 100 mV, which is almost same as that of non-carbon-coated membrane without irradiation of electron beam. Charges induced by electron beam were escaped to the ground through the high electrically conductive thin carbon layers deposited on the membrane windows.

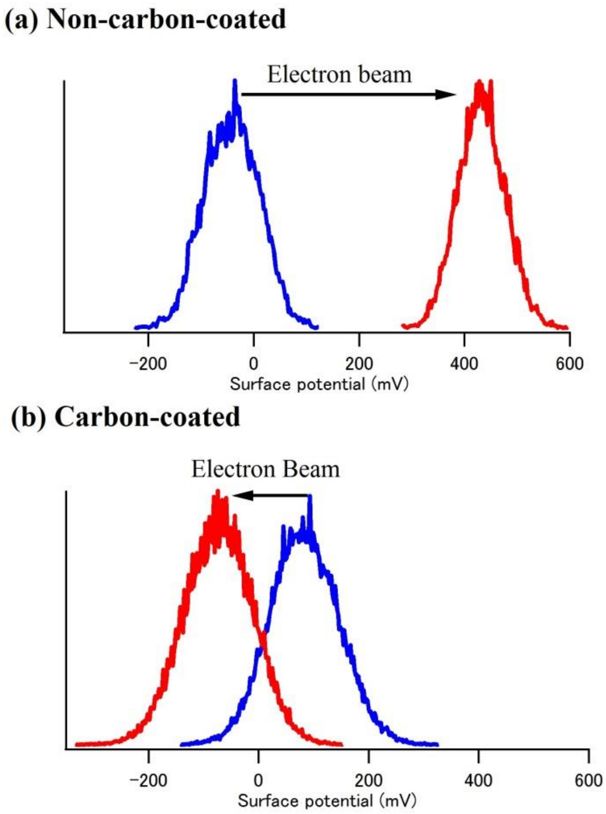

Figure 1: Surface potential of (a): non-carbon-coated and (b): carbon coated membrane (thickness: 50 nm). Each blue and red lines represent before and after irradiation of electron beam (10 pA/cm^2 for 10 min), respectively.

References

[1] U. Mirsaidov, H. Zheng, D. Bhattacharya, Y. Casana, P. Matsudaira, Proc. Natl. Acad. Sci. U.S.A. 109(19), 7187-7190 (2012).

[2] H. Zheng, U. Mirsaidov, L.-W. Wang, P. Matsudaira, Nanoletters, 12(11), 5644-5648 (2012).

[3] U. Mirsaidov, C. D. Ohl, P. Matsudaira, Soft Matter 8(27), 3108-3111 (2012).

[4] U. Mirsaidov*, H. Zheng*, Y. Casana, P. Matsudaira, Biophysical Journal 102(4), L15-17 (2012).

[5] R. Danev, K. Nagayama. Ultramicroscopy 88, 243-252 (2001).

[6] A. C. Milazzo, et al. Ultramicroscopy 110 (7), 744-7 (2010).