Temporal behavior or interaction of biomolecules is generally observed by a light microscope (LM). However, resolution of the LM is insufficient to resolve fine structural details. Although the location of target molecules can be recognized by fluorescent labeling with a fluorescent microscope (FM), other molecules or structures which are unlabeled cannot be observed in detail by an LM. In contrast, a transmission electron microscope (TEM) has much higher spatial resolution than LM. However, disadvantages of TEM include inability to observe dynamics of specimens with bioactivity and paucity of labeling methods. Correlative light and electron microscopy (CLEM) is a bridge between the two microscopic methods. The CLEM gives us advantages of both TEM and LM which are a broad overview of the specimen and temporal events with LM and high-resolution information about the specimen with TEM.

We developed a workflow for the CLEM. The workflow utilizes a piece of software named "picture overlay program", which shares the coordinates of TEM and LM images. The software shares the coordinates automatically, with indication of the two corresponding points in each image. The correlation of LM and TEM, which is optimized for observation of a biological sample, is realized after the sharing. The TEM used for our experiments was chosen to be JEM-1400Plus (JEOL Ltd., Japan). The correlation is realized by using a function named “stage navigation". The “stage navigation” is realized with the “ultra low-magnification” and easy-to-use specimen drive method named “point & shoot”. The lowest magnification for the microscope is 10 x. With that magnification, a whole TEM grid can be observed in a single field of view. “Point & shoot” is a method which points the next desired destination by a mouse click and makes the point to the center of the field of view by the specimen motor-driving system of the TEM stage.

In the workflow, first, a coordinates of LM image is shared with the corresponding ultra low-magnification TEM image by the “picture overlay program”. After the sharing of the coordinates, one can center a region as one desires by the “point & shoot” (Fig.1). And then, users can increase the magnification to see a detailed structure of the specimen. In addition, the “picture overlay program” can save a multi-layer image (Fig.2). The each layer has the detailed structural information by TEM or functional information by FM or LM. Thus, by TEM, users can observe regions of interest which are marked with LM and/or FM with high resolution. This workflow is helpful for CLEM applications.
Fig. 1: Screenshot of windows for CLEM, running software: “picture overlay program” on a TEM operation monitor. TEM and LM images are displayed simultaneously. The software superimposes the images and shares their coordinates of them. With the shared coordinates, the TEM stage can move automatically to a region of interest found in the LM image.

Fig. 2: Superimposed images of LM, FM and TEM by using the “picture overlay program”. The specimen were chemically fixed and stained by Hoechst 33342.