We have developed a contact soft x-ray microscope combined with an intense laser-plasma soft x-ray source to achieve flash imaging of live hydrated biological cells. Laser-plasma soft x-ray source produced by a high power pulsed laser is extremely bright and very suitable for biological x-ray microscopy to capture an image of living specimens for which require a single flash exposure to avoid imaging any damages on the specimens. We also have invented to use a fluorescent microscope to identify the cellular organelles in the images obtained with the soft x-ray microscope. The biological cells were cultivated directly onto the PMMA photo resists and observed with the soft x-ray microscope and the fluorescent microscope at the same time. The obtained soft x-ray images and fluorescence images of the cells were directly compared and each cellular organelle such as mitochondria, actin filaments, and chromosomes in the soft x-ray images were clearly identified. Since the soft x-ray microscope has higher spatial resolution than that of the fluorescent microscope, fine structures of the cellular organelles in the hydrated biological cells were observed.

Shown in figure 1 are the soft x-ray image (a) and the fluorescence image (b) of the live biological cells. Appearing blue in the fluorescence image were chromatin, red were mitochondria, and green were actin filaments. The both images were clearly identical and each cellular organelle in the soft x-ray image could be identified directly comparing with the fluorescence image.

Shown in figure 2 are the soft x-ray images of one of the cells (a) shown in Fig.1 and enlarged images of surrounding area of the nucleus (b) and mitochondria (c) in the same cell. The cellular organelles such as chromatin and mitochondria in the images were identified comparing directly with the fluorescence image. All of the bright spots surrounding the nucleus in Fig. 2(a) were recognized to be mitochondria. Shown in Fig. 2(c) is the soft x-ray image of a single mitochondrion picked up from the Fig. 2(b) and detailed structure of the mitochondrion was obtained.

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Fig. 1: Soft x-ray image (a) and fluorescence image (b) of live hydrated biological cells. Appearing blue in the fluorescence image were nuclei, red were mitochondria and green were cytoskeletons.

Fig. 2: Soft x-ray images of one of the cells (a) shown in Fig. 1 and enlarged images of surrounding area of the nucleus (b) and mitochondria (c) in the same cell. The cellular organelles such as chromatin and mitochondria in the images were identified comparing directly with the fluorescence image.