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Subcellular Protein Localization with Hard X-Ray Microscopy

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We present a novel imaging technique which uses hard x-rays to image the sub-cellular structure with a 60 nm resolution. The key factors to our success are the use of a coherent synchrotron sources in conjunction with a high definition phase zone plate lens in x-ray microscope [1] and suitable protein-labeling techniques. By using the zone plate lens to magnify the projected x-rays, we produced high-quality images of cells and yeast with a 60 nm resolution. This technique is not limited to 2-dimensional images. Tomographic data could be obtained through limited angles reconstruction, which provides 3-dimensional information of specimens, including organelle structures, protein distribution, cell location and morphology of multi-cellular structure.

We found immunostaining, a common method for labeling proteins, to localize the intermediate filament vimentin is quite suitable for high resolution x-ray microscopy. To increase the contrast for x-ray microscopy, DAB with nickel enhancement staining kit was used. The DAB/nickel-labeled vimentin networks and the individual bundles with diameters less than 100 nm can be observed. As shown in FIG. 1., by labeling the amyloid-like Sup35 protein aggregations [2] we also successfully imaged the Sup35 Green Fluorescence Protein fusion protein aggregates inside yeast with the size less than 100 nm again stained by DAB/nickel. Here the GFP-tagged yeasts were labeled by anti-GFP antibody.

To explore the structure, distribution, and translocation of proteins and organelles inside cells, 3-dimensional information were achieved, as shown in FIG. 2, by tomographic reconstruction with limited angle . The cell sample stained by immunogold with silver enhancement was placed on a holder which is rotated from -70 degrees to 70 degrees with 1 degree step. The different colors represent different parts of the cell which are identified by their x-ray contrast. Specifically, the small dots (gold color) are those from the heavy absorbing metallic nanoparticles aggregate.

Reference

[1] W. Yun et al., Rev. Sci. Instrum. 70 (1999) 3537.
[2] C. Y. King, J. Mol. Biol. 13 (2001) 1247.

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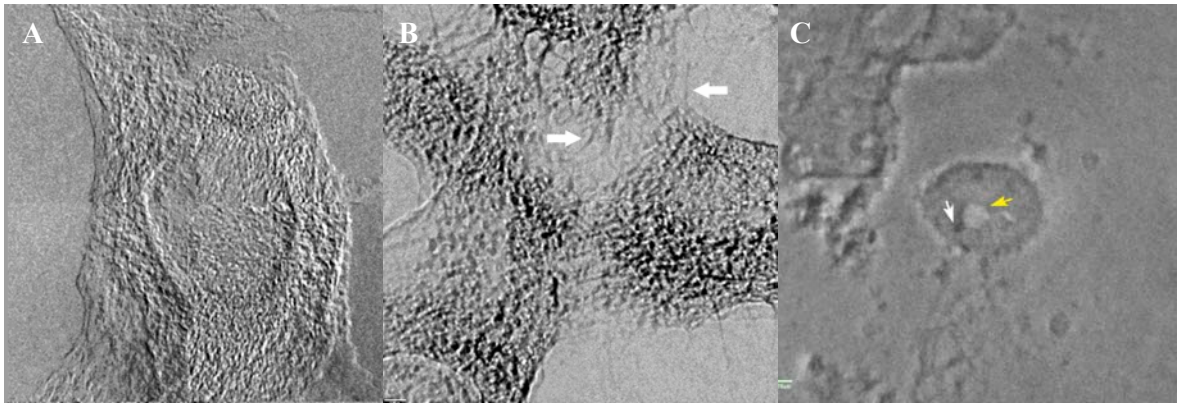

FIG. 1. A. The vimentin filaments inside HeLa cells were stained with DAB/Nickel and photographed by X-ray Microscopy. B. The same specimen as A. Image was taken with a Zernick phase ring producing enhanced phase effect. Arrows indicate the individual vimentin bundles with the diameter less than 100 nm. FOV = 30 μm. **C.** The dark spots indicated by white arrows are the Sup35 GFP fusion protein aggregations stained by DAB/nickel. The yellow arrow indicates the vacuole which is less dense. FOV= 15μm.

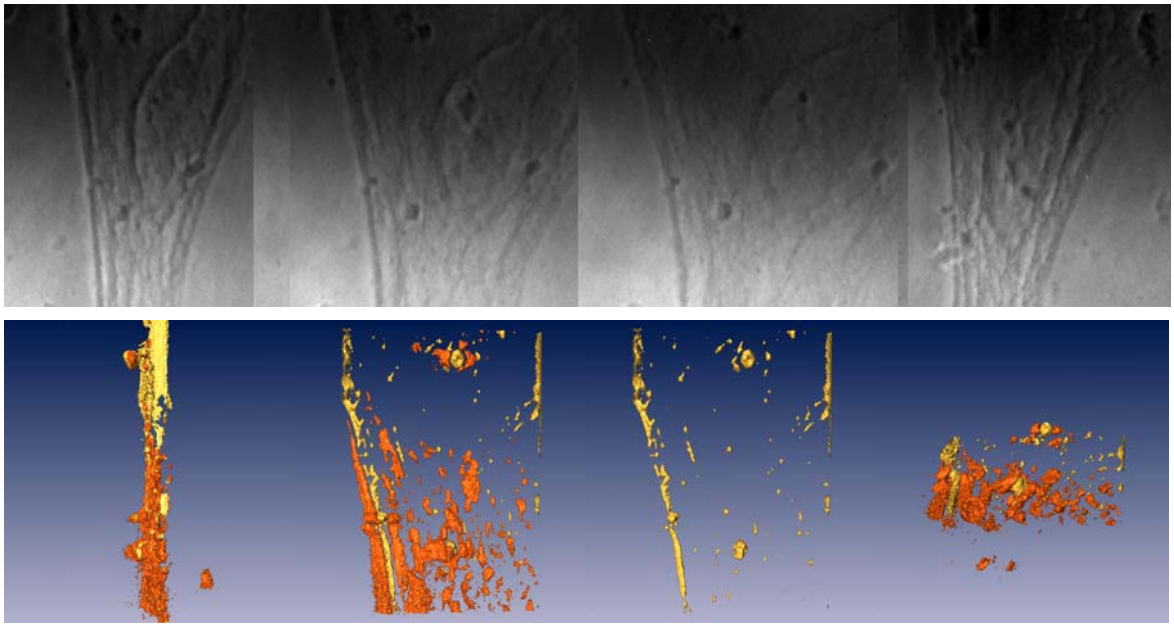

FIG. 2. Tomographic data of the HAEC cell stained with immunogold and silver enhancement collected by X-ray Microscopy. Upper images show sequential images taken from different angles. Lower images are 3-dimentional reconstruction images from different angles of projections. The different parts of the cells were indicated by different colors based on their absorption contrast. FOV of each frame is 15μm.

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