

Imaging Sub-cellular 3D Structures Using Soft X-ray Microscopy

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We are using soft x-ray microscopy (SXM) to image and quantitatively study the three dimensional (3D) sub-cellular structures within single cells. How the alternations of cellular morphology as well as the organelles' 3D spatial distribution and re-organization are crucial for cell biologists to answer questions that may relate to the mechanisms of some diseases, such as cancer metastasis, diabetes or bacterial invasions. Soft x-ray tomography (SXT) is a non-invasive label-free technique with high spatial resolution up to 35nm (1). It is a quantitative image technique that the high contrast images were generated by the absorption of soft x-ray to different compositions of bio-organic materials within intact cells in their near-native state. Using soft x-ray as an illuminating light source takes advantage of "water-window" that the contrast differences come from distinct cell constituents. The absorption is also linear with the concentration of bio-organic molecules. Each voxel in the sub-cellular structure has a unique value called "linear absorption coefficient (LAC)" that can be used as fingerprints for each organelle. For example, lipid droplet has the LAC of about 0.7 (μm^{-1}) while mitochondria's LAC is about 0.33 (μm^{-1}) (2).

We have used different concentrations of collagen to reconstitute a 3D microenvironment inside the capillaries, the sample holder, which has different pore sizes for cancer cells (HT1080) to crawl through. We have observed that the cells have different morphologies with different physical limitations created by collagen polymerization. We can also quantitatively study the variations in nuclear size and shapes, the 3D distributions and volumes of mitochondria and lipid droplets and analyze the ratios of between euchromatin and heterochromatin(3). SXT has also been used to determine the ultrastructural architecture of beta-cells which will be ultimately used to build a whole single-cell model. X-ray tomograms can be used to extract information such as organelles' distribution, quantity, shape, and size with the function of chemical stimulations (4). The information will be key to establish the spatial and temporal models to map various reactions of beta-cells under different stimulating conditions. In addition, we have used SXT to examine nucleoid genetic material from *E. coli* with minimal perturbations to the cells. We examined the nucleoid variations between wild-type *E. coli* and HU-Alpha mutant *E. coli* and found that altering the HU-Alpha protein made the bacteria more invasive and triggered condensation in all three growing phases(5).

References

Reference:

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