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Soft X-ray Spectromicroscopy and its Potential for Biological Applications

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Abstract

Light reflectance, transmission or scanning electron microscopy is often used to determine the structural characteristics and chemical distributions of biological objects. These techniques have the limitations of low spatial resolution and chemical sensitivity or extensive sample preparation methods.

Soft X-ray spectromicroscopy is a spectroscopy and microscopy technique that utilizes the bright and coherent X-rays produced at Synchrotrons. It can characterize substances at the nanometer scale based on the absorption edges of elements present. This makes it an ideal tool for biological applications where structural and chemical characterization is highly desirable at a high spatial resolution. The principle of soft X-ray spectromicroscopy, features of soft X-ray microscope at the Canadian Light Source, and the potential of soft X-ray spectromicroscopy for biological research is presented in this paper.

1. INTRODUCTION

A good understanding of the structural and chemical composition of biological products and of the mechanisms of their interactions with the ecosystem is essential to preserve the qualities and to develop effective solutions for the problems. Cells are the building blocks of plants and animals and the structural and chemical composition of cells and organelles, if resolved to the finest scale, can lead to tremendous advancement. The size of plant and animal cells and bacteria are at micrometer scale whereas the size of virus and molecules are in the nanometer range (Figure 1).

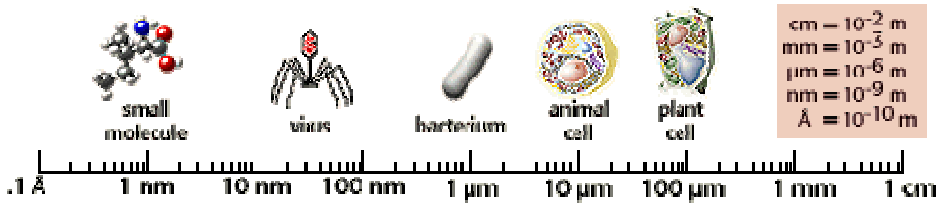


Figure 1. Relative size of cells, microbes, and molecules (Source: Anonymous 2006a).

The wavelength of light used to 'see' an object must be equal or smaller than the size of the object. The spatial resolution depends on the wavelength of light used and chemical sensitivity depends on access to the wavelengths specific to the material of interest. Often visible light microscopes used in reflectance, transmission, phase contrast, or fluorescence modes can resolve structures at the micrometer spatial resolution which is limited by the wavelength of visible light (Figure 2).

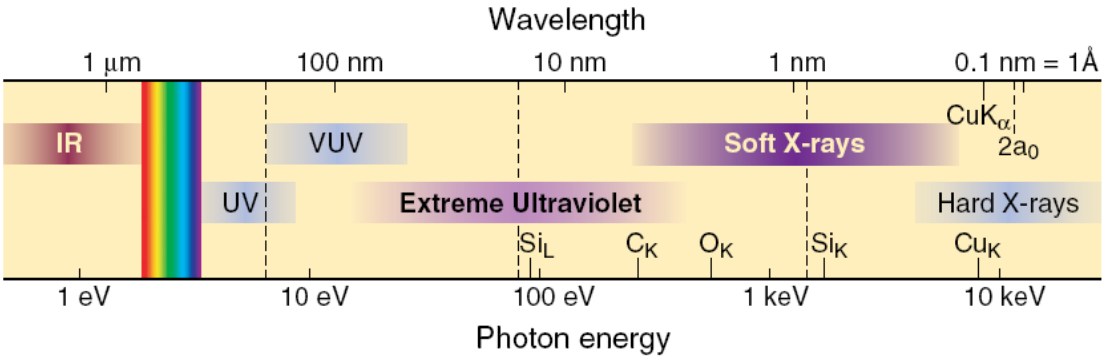


Figure 2. Electromagnetic spectrum (Source: Anonymous 2006b).

Alternatively, electron microscopes in the transmission and scanning modes are used to resolve cell structures at a spatial resolution of up to 2 nm. However, electron microscopes cannot be used to study wet cells and require extensive sample preparation such as embedding in resin and slicing to ultra thin sections of less than one hundred nanometers and sometimes requires metal coating (Lawrence et al. 2003).

Visible light microscopy does not provide any information on the chemical composition. The chemical sensitivity of electron microscopy is very low and there is ambiguity whether the information is due to chemical changes or density variations of the specimen

(Koprinarov and Hitchcock 2000). In addition, electron microscopy produces much larger radiological damage for a given amount of analytical information. Such damage is often a critical factor in studies of biological products (Kirz et al. 1995).

Infrared, nuclear magnetic resonance, and Raman spectroscopy presently used for chemical characterization of biological products have spatial resolutions of micrometers. Advanced laboratory-scale systems utilizing electromagnetic radiation in the infrared, visible, ultraviolet, soft X-ray, and hard-X-ray regions of the spectrum are used to explore the physical and chemical constituents of biological products by generating images, spectra, or combinations of both (Anonymous 2006c). The lab-based systems have limited spatial resolution and low elemental and chemical sensitivity since they are restricted to single wavelength or narrow ranges and cannot be easily tuned over a wide range of the electromagnetic spectrum. For instance, the spatial resolution of objects imaged with infrared energy is lower than with soft X-rays as the wavelength of infrared rays (4-10 μm) is higher than the soft-X-rays (1-10 nm). Within soft X-rays, the wavelengths must be tuned to narrow bands to probe the presence of different elements or chemicals in a product. This paper highlights the synchrotron light source and the advantages of using soft X-rays from a synchrotron light source for biological imaging.

2. WHAT IS A SYNCHROTRON?

Accelerated charged particles traveling in a curved trajectory in the presence of a magnetic field emit radiation tangentially to the path (Attwood 1999; Anonymous 2006d). Synchrotrons are based on the same principle and are the sources for extremely bright, collimated, and focused light in the ultraviolet to hard X-rays. This allows one to investigate objects at high spatial resolution and chemical sensitivity at the atomic to molecular level (Anonymous 2006e). Figure 3 shows the schematic of a synchrotron (Attwood 1999). The electrons traverse in magnetic field structures of different magnetic strength in the straight sections as shown in the figure. The greater the magnetic field, the angular excursion by the electrons are high and produces long wavelength hard X-rays. The magnetic field strength for soft X-rays is smaller than for hard X-rays. The brightness of synchrotron light provides the additional advantages of achieving higher spatial as well as spectral resolution from the sample. Up to now, synchrotron light has mainly been used for research in materials science, medical imaging, and protein crystallography.

The Canadian Light Source Inc. (CLS) at Saskatoon is one of the new generations of synchrotrons and the only one in Canada that was opened in 2004. The CLS has infrared, soft and hard X-ray source beamlines for various types of research applications (Anonymous 2006d).

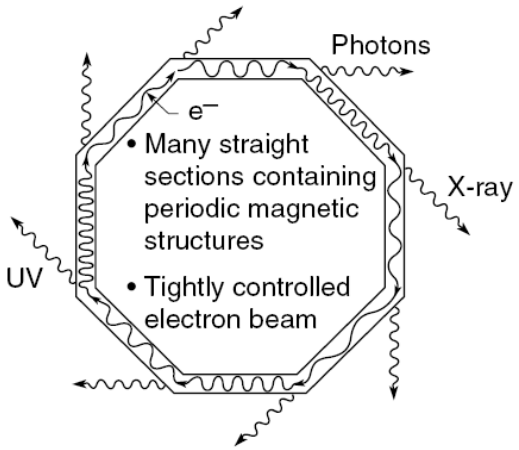


Figure 3. Schematic of a synchrotron light source (Source: Attwood 1999).

3. SOFT X-RAY ABSORPTION SPECTROSCOPY

Soft X-rays are electromagnetic radiations between the ultraviolet and hard X-rays. The relationship between wavelength and photon energy is given by (Attwood 1999, Figure 2):

$$\lambda = \frac{hc}{E} \qquad (1)$$

where: λ = wavelength (nm);
h = Planck's constant (4.1357×10^{-15} eV.s);
c = speed of light in vacuum (2.9979×10^{8} m.s^{-1}); and
E = photon energy (eV).

As the energy of light increases towards the X-ray region, the wavelength decreases that helps to achieve a higher spatial resolution. Soft X-rays have longer wavelength and lower photon energy than hard X-rays. The wavelength of hard X-rays in the Armstrong range is utilized to study the inter-atomic distances of molecules using X-ray diffraction techniques. Soft X-rays on the other hand are used to determine the chemical composition of samples by studying the chemical bonding and valence bond structures (Smith 2001). The low energy of soft X-rays makes this band ideal for biological imaging, as low density materials are sensitive to radiation damage. Soft X-rays from the synchrotron light sources has the advantage of being tunable to get monochromatic illumination of specific wavelengths than the laboratory scale systems. The monochromatic X-rays are particularly helpful to probe the binding energies of core electrons that are characteristics of physical and chemical state of the atoms.

When X-rays interact with matter, part of the incident photon is absorbed and the remaining is transmitted through the object. The absorbed photons cause excitation of the inner shell electrons of the atoms and the absorption is characteristic of the object such as the object density and thickness (Figure 4). The excited electrons can be either removed completely from the atom (ionization) or promoted to unoccupied shells in the

atom (excitation) (Koprinarov and Hitchcock 2000).

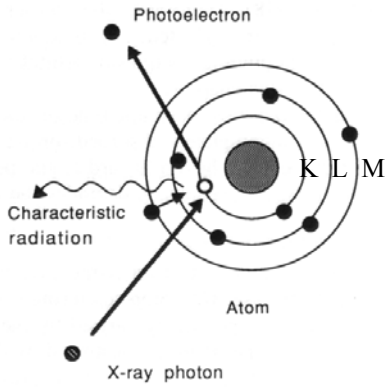


Figure 4. Schematic on the interactions of soft X-rays with matter (Source: Anonymous 2006f).

X-ray absorption spectroscopy is based on the ionization of inner shell electron called absorption edges which are element specific. For instance, the binding energies of the one core level (K-shell) electron of carbon, nitrogen, and oxygen are 290, 400, and 530 eV (Smith 2001). Monochromatic X-rays from 250 to 1000 eV energy can be used to probe K-shell electrons of C, N, O and K and L-shell electrons of the first row transition metals in the periodic table (Figure 5). The energy region between carbon and oxygen is called the "water window" which provides higher contrast that is attractive to image wet and biological samples. In the "water window", water is transparent to soft X-rays whereas soft X-rays are highly absorbed by carbon (Kirz et al. 1995).

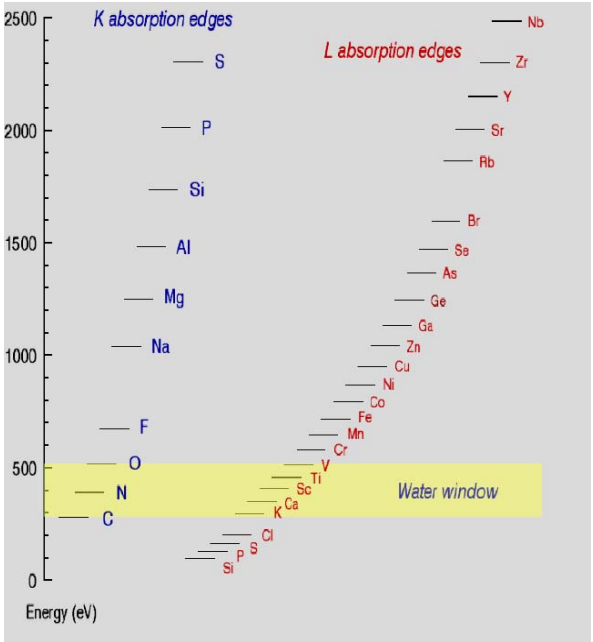


Figure 5. Soft X-ray absorption energy levels from 100 to 2500 eV of different elements (Source: Anonymous 2006g).

4. SOFT X-RAY SPECTROMICROSCOPY

Spectromicroscopy is based on X-ray absorption spectroscopy that not only provides spectroscopic information from a small spot in the specimen but can also provide spatial distribution of elements at the nanometer resolution (Figure 6). For instance, the absorption spectra of DNA and bovine serum albumin are different near the carbon absorption edge due to the difference in the bonding states of carbon. Scanning of the specimen at the different absorption edges provides a spatial map of their distribution, for example the DNA distribution in the specimen.

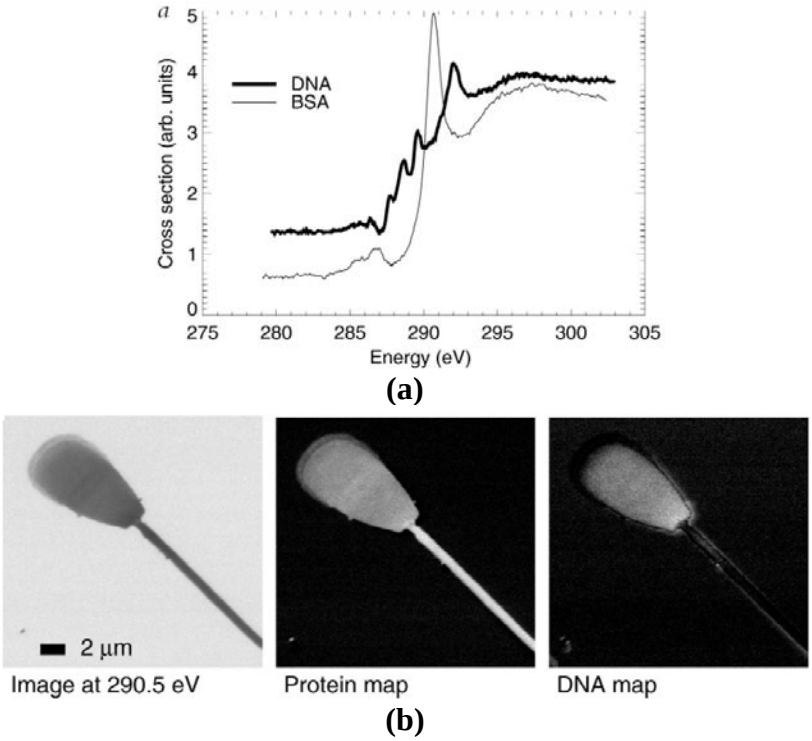


Figure 6. Absorption Spectra of DNA and bovine serum albumin (a) and protein and DNA distribution in bull sperm (b) (Source: Jacobsen and Kirz 1998).

The two common techniques of soft X-ray microscopy are the photon-in-photon-out and photon-in-electron-out modes. In the photon-in-photon-out mode, the energy of the transmitted x-rays is used to determine the composition of the specimen while in the photon-in-electron-out mode the energy of the ejected electron from the specimen is analyzed. The photon-in-electron-out technique is surface sensitive and wet specimens cannot be analyzed using this technique.

Figure 7 shows a schematic of the soft X-ray beamline at a synchrotron that provides transmission soft X-ray spectromicroscopy. The undulator is a special type of insertion device or magnetic structure in the new generation synchrotrons that provides bright and coherent photon beams for soft X-ray applications research. The monochromator is used to select the element specific energy of the X-ray beam used to probe the specimen. The monochromatized light is then focused on the specimen using a zone plate which

collimates the beam through a diffraction order sorting aperture (OSA) to generate a spot size of about 50 nm. The spatial resolution achieved is based on the resolution zone plate used. To acquire spectral information, the X-ray beam is focused on the specimen and the monochromator is scanned to provide an X-ray beam with different energy X-ray levels. For obtaining the distribution of chemicals in the specimen, the position of the monochromator is fixed at a selected X-ray energy and the sample is raster scanned and the transmitted X-rays are recorded. The soft X-ray spectromicroscopy beamline at the Canadian Light Source Inc. is designed to provide monochromatized X-rays from 100 to 2000 eV and the X-rays can be polarized from circular to linear inclined angles (Anonymous 2006d).

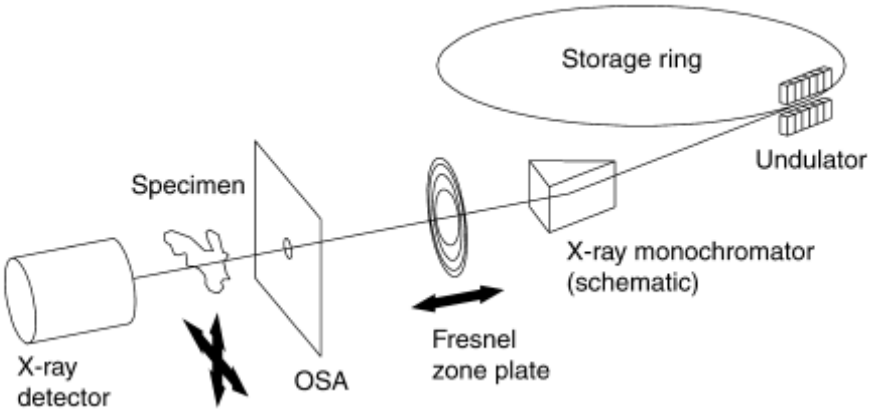


Figure 7. Illustration of a soft X-ray spectromicroscopy beamline (Source: Maser et al. 2000)

Unlike electron microscopy, soft X-ray spectromicroscopy has the advantage to study biological material in their natural forms, wet or dry and with much less radiation damage. The comparative advantage of achieving higher spatial resolution using soft X-ray relative to visible light imaging is evident from Figure 8.

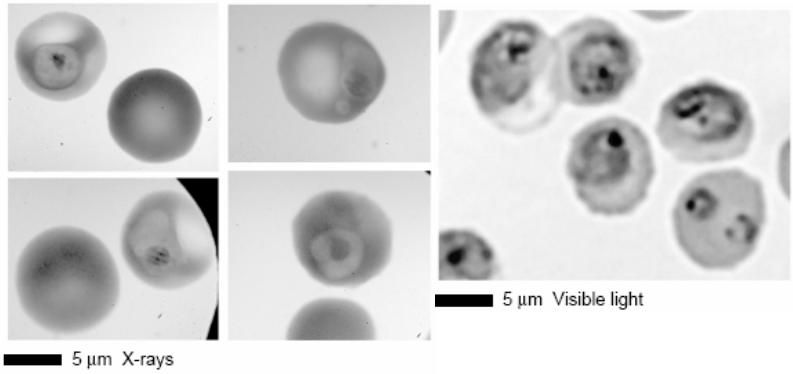
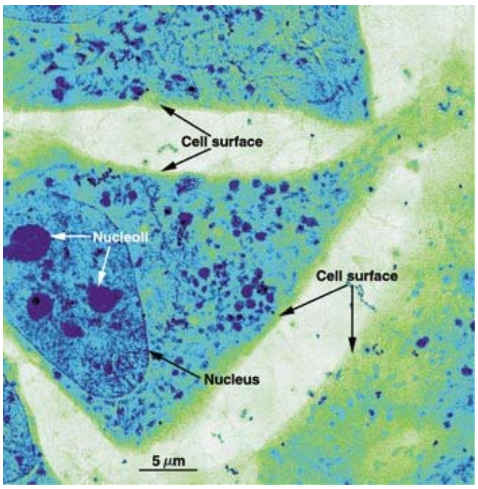
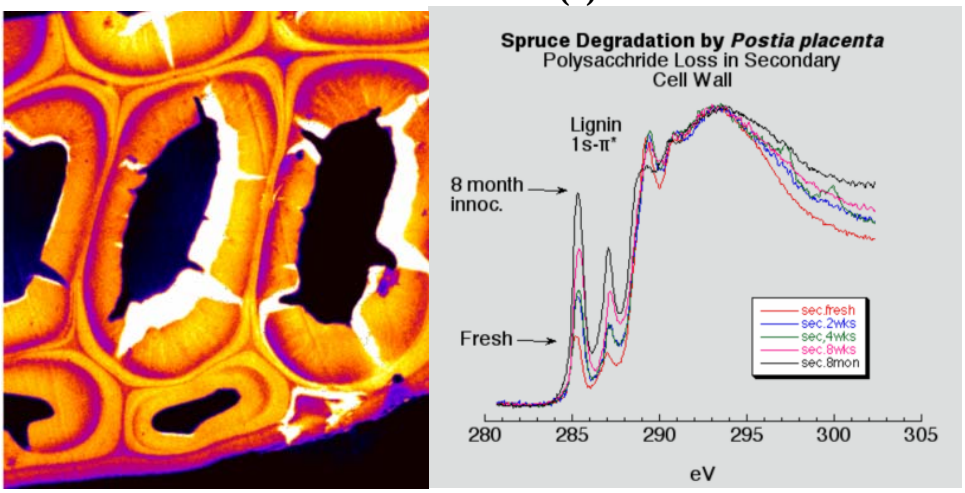


Figure 8. Comparison between visible light and soft X-ray images of human erythrocytes infected with *P. falciparum* (Source: Kirz 1995).

Figure 9 shows a few examples of using X-ray microscopy for biological imaging. The chemical and structural composition of plant and animal cells helps to understand the functional mechanism and to develop effective drugs for the treatment and prevention from diseases. The distribution of lignin in plant cells helps to develop species with a higher resistance against microbial attack and to reduce crop loss in the agricultural and wood industry. The imaging of specimen in three dimensional space is possible with soft X-ray spectromicroscopy. Figure 9c shows a three-dimensional reconstructed yeast cell embedded with gold particles. The gold particles are embedded to better align all the image slices as the specimen is rotated during imaging. Figure 8d shows an example on the study of mineralization of bacteria in a biofilm using soft X-ray spectromicroscopy.



(a)



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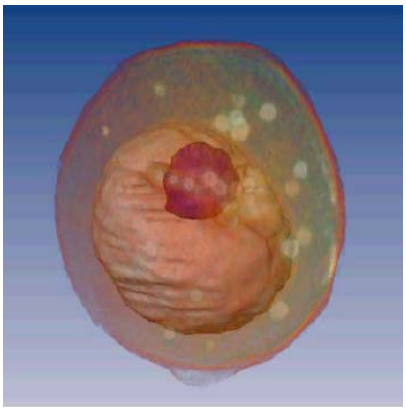
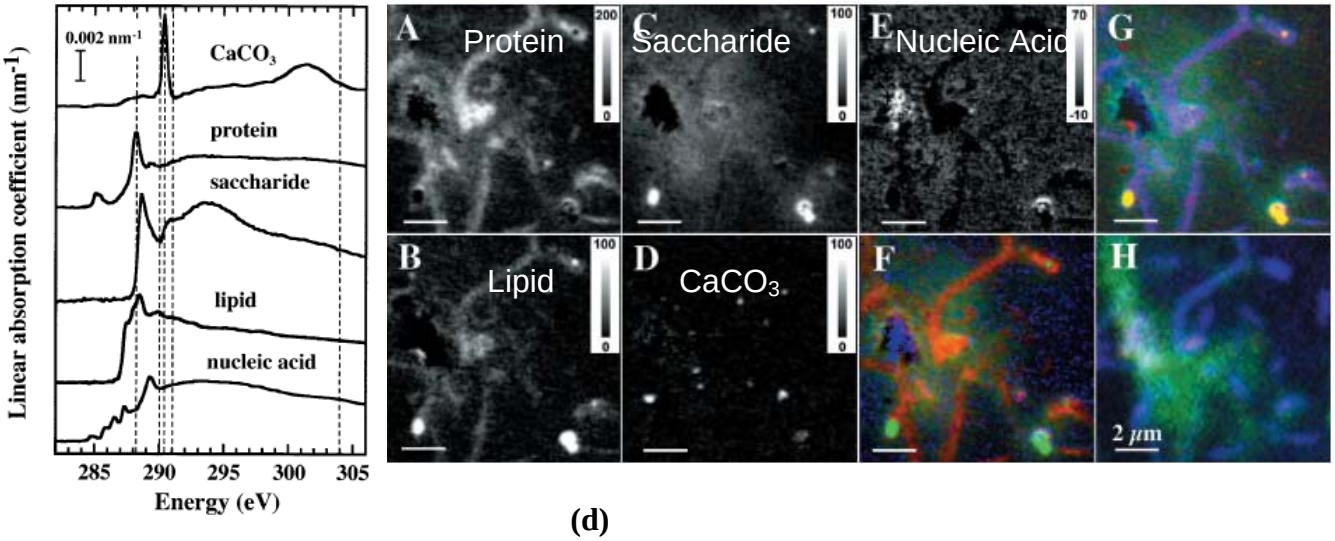


(c)



(d)

Figure 9. False color coded Soft X-ray transmission images of connective tissue cell from a mouse (a), distribution and degradation of lignin in plant cells (b), tomography of yeast cell (c), and mineralization by biofilms) (Sources: Anonymous 2006e; Anonymous 2006h; Larabell and LeGros 2004; Lawrence et al. 2003).

5. CONCLUSIONS

Soft X-ray imaging provides the advantages to visualize objects at a much higher resolution with the visible light. The resolutions of soft X-ray images are lower than electron microscopy. However, soft X-rays provide chemical compositional information and produce less radiological damage to the specimen than the electron microscopy. This makes it an attractive technique for biological studies. Synchrotron based soft X-ray spectroscopy and imaging has the advantages of brightness, tunability, and coherence which provides a unique advantage for the advancement of research in biological science.

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