X-ray microscopy and biological applications

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Abstract – Improvements in X-ray microscopy instrumentation, automation and new generation of cryo sample stages are opening the door to biological applications. The complementary character of X-ray microscopy with regards to confocal and electron microscopy can make of this technique a useful tool to address a certain number of biological problems. However, it is mandatory a combined effort between the X-ray physics and biological communities.

1. Introduction

After a half century of development, X-ray microscopy (XRM) has cleared a technological path to complement light and electron microscopies (EM) in the biological area. Its combination of resolution, penetrating power, analytical sensitivity, compatibility with wet specimens and the ease of image interpretation has turn it into a useful tool also for imaging frozen hydrated biological specimens similarly to transmission electron microscopy.

Transmission X-ray microscopes (TXM) specialize in the rapid acquisition of 2D images and in the collection of tilt sequences of projections for tomography reconstruction using high flux. Scanning transmission X-ray microscopes (STXM) focus on the acquisition of reduced dose images and point spectra with high energy resolution for elemental and chemical mapping thanks to the high brightness of synchrotron radiation sources.

Modern XRM is based on two key points: 1) the water window energy range, between the inner-shell absorption edges of carbon and oxygen where water layers up to 10 μm can be penetrated whereas organic cell structures are visualized with good absorption contrast, and 2) microfabricated zone plate lenses, which are used as condensing and objective lenses. In addition to the water window, it is worth mentioning that the 1.5-3.0 keV region, giving less absorption and more phase contrast, is interesting as well to overcome limiting factors intrinsic to the use of zone plates in TXM (*i.e.* the depth of focus and the focal length).

Early TXM and STXM were capable of 2D imaging of wet samples at room temperature, which fit the needs of polymer and environmental sciences, for example. But a mandatory requirement for biological applications is that imaging has to be performed below the critical level for radiation damage. Estimations of the radiation dose required to image 20-nm-thick protein features in various ice thicknesses as a function of the X-ray energy are shown in Figure 1 [1]. The critical dose for imaging frozen hydrated biological material at typical XRM resolution is about 10^9 Gray.

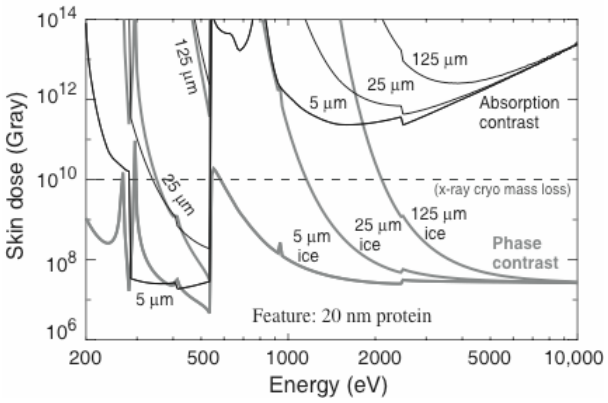

Figure 1- Radiation dose calculated for minimal exposure (S:N=5:1) of 20 nm protein structures in different ice thicknesses for amplitude and phase contrast in function of the X-ray energy; 100% efficient optics and detectors are assumed.

Until the use of cryogenic temperatures to avoid mass loss, tomography of biological samples was therefore not possible. Nowadays, utilizing the improvements in cryo sample stages achieved by the EM community, XRM can fill the gap between light and electron microscopies in spatial resolution and sample thicknesses.

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2. First examples of biological applications

The scientific breakthrough in biology using X-ray microscopy has not yet occurred, but the instrumentation to make it possible is now coming into existence. It has been proven that TXM can give insight into the internal structure of whole cells as specimens can be imaged in their natural hydrated state without using stains and sectioning methods, and with reasonable spatial resolution. 2D images at 35 nm of spatial resolution were obtained in 1998 by G. Schneider [2], among others, and cryo 3D reconstructions of yeasts and bacteria at 60 nm were achieved by C. A Larabell *et al*. [3] supporting in 2004 the "proof of concept" of this novel technique.

New TXMs with cryo sample stages are right now under construction or commissioning, with performance and automation that would allow collecting a complete data set for tomography in several minutes at significant improved resolution (around 30 nm) and biological significant statistical numbers.

In addition, efforts in the manufacture of zone plates with smaller outer-most zone width, determining the spatial resolution, have been promising. Indeed, 15 nm was reached in 2005 by Chao *et al.* [4]. However, as a new technique in the biology area, the development of new labels that can be visualized by absorption contrast to target specific cell sites will be mandatory as it was for confocal and electron microscopies.

3. Conclusions

X-ray microscopy for biological applications has now overcome the step of "proof of concept". Combined efforts of the X-ray physics and biological communities have to continue so that this novel technique can become a useful tool, complementary to confocal imaging and transmission electron microscopy. The number and type of biological problems that can be addressed with X-ray tomography is expanding, among them structural studies, and localization of subcellular components in cellular events. Overall, X-ray microscopy is coming of age.

4. References

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