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Review

X-ray microtomography in biology

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ABSTRACT

Progress in high-resolution X-ray microtomography has provided us with a practical approach to determining three-dimensional (3D) structures of opaque samples at micrometer to submicrometer resolution. In this review, we give an introduction to hard X-ray microtomography and its application to the visualization of 3D structures of biological soft tissues. Practical aspects of sample preparation, handling, data collection, 3D reconstruction, and structure analysis are described. Furthermore, different sample contrasting methods are approached in detail. Examples of microtomographic studies are overviewed to present an outline of biological applications of X-ray microtomography. We also provide perspectives of biological microtomography as the convergence of sciences in X-ray optics, biology, and structural analysis.

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Contents

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1. Introduction

Progress in high-resolution X-ray microtomography (also known as microcomputed tomography or micro-CT) has provided us with a practical approach to determining the three-dimensional (3D) structure of an opaque sample at micrometer to submicrometer resolution (Bonse and Busch, 1996; Salomé et al., 1999; Uesugi et al., 2001; Takeuchi et al., 2002). Applications of X-ray microtomography have been reported for a wide variety of objects (Toda et al., 2008; Chen et al., 2009; Fusseis et al., 2009; Tsuchiyama et al., 2011; Zhu et al., 2011). In biology, X-ray microtomography has revealed 3D structures of biological samples from many species of organisms including human (Bonse et al., 1994; Salomé et al., 1999; Mizutani et al., 2008a), mouse (Johnson et al., 2006; de Crespigny et al., 2008; Mizutani et al., 2009a), and insect (Mizutani et al., 2007; Metscher, 2009; van de Kamp et al., 2011). It has become a common method in studies associated with osteo and dental microstructures (Neues and Epple, 2008; Zou et al., 2011). Microtomographic studies of soft tissues, which account for a major proportion of biological tissues, have shed light on the structural mechanism of biological functions (Happel et al., 2010; Mizutani et al., 2010a).

The constituents of soft tissue are cells and extracellular matrices that are responsible for biological functions. For example, the cellular structures of brain tissue or subcellular structures of nucleus are essential for their activities. In this review, we focus on hard X-ray microtomography of biological soft tissues at cellular to subcellular resolution, i.e., micrometer to submicrometer resolution. The subcellular and cellular microstructures build up a 3D structure, called tissue, with dimensions of the order of hundreds of micrometers.

Although microscopy using visible light is the primary method for visualizing structures of biological systems, absorbance and refraction of the visible light interferes with the determination of the internal structures of real biological objects and this interference increases with tissue thickness. Thus, light microscopy is mainly used for imaging sectioned samples. By contrast, the transmissive and less refractile nature of hard X-rays with respect to biological tissue enables radiographic observation of the 3D structure. This allows microtomographic studies of the 3D structure of biological objects that have not been visualized before.

Below, we discuss the fundamentals of X-ray microtomography in biology for those who are not familiar with microtomography. Practical aspects of biological sample preparation, handling, data collection, 3D reconstruction, and structure analysis are described. Examples of microtomographic studies are overviewed to present an outline of biological applications of X-ray microtomography. In the last section, we give perspectives of X-ray microtomography in biology as the convergence of sciences in X-ray optics, biology, and structural analysis.

2. Microtomography in practice

2.1. X-ray visualization of biological tissue

Since X-rays interact with electrons in sample objects, electron-dense or electron-poor structures can be visualized in an X-ray image as contrast against the background. However, biological soft tissue is composed of low-atomic-number (low-Z) elements, such as carbon and oxygen, which produce little contrast in a hard X-ray image. Most biological soft tissues exhibit uniform density in a conventional X-ray image in which only the outline but no internal constituents are visualized. Although the structural outline is biologically relevant in some cases, the internal structure is crucial for the biological function of soft tissue.

In order to visualize soft tissue effectively, we should label the structure of interest with a probe appropriate for the observation method. In modern biological studies using light microscopy, the standard procedure for visualizing the target structure involves labeling the sample with a visible dye or fluorescent probe. Similarly, X-ray-specific labeling should be performed for X-ray visualization except in circumstances in which sample pretreatment is not feasible such as in the case of fossils (Friis et al., 2007). In clinical diagnosis, a possible visceral lesion behind the skin is examined using X-rays. Although soft tissues of the human body give little contrast in an X-ray image, luminal structures can easily be visualized by using X-ray contrast media. These contrast media contain high-atomic-number (high-Z) elements, such as iodine or barium, which absorb X-rays efficiently. In the same way, X-ray visualization of the microstructures of soft tissues can be performed by specifically labeling each biological constituent with a high-Z element probe. A number of labeling methods in biological microtomography have been reported, as overviewed in the following section.

In X-ray microtomography, the interaction of X-rays with a sample object is mostly visualized by one of three types of modality: absorption, phase interference, or fluorescence. Which of these modalities should be used for visualizing samples labeled with high-Z element probes? The X-ray fluorescence of a probe element down to the concentration of 1 ppm can be visualized three-dimensionally (Lanzirotti et al., 2010), although it takes much more time to acquire a fluorescence image compared with the usual X-ray image. Hence, the application of fluorescence microtomography is still limited to a small number of examples at present. Microtomographic studies using phase contrast methods have been reported for a number of biological samples (Betz et al., 2007; Connor et al., 2009; Wu et al., 2009; Schulz et al., 2010). However, the prerequisite for these applications is that the sample objects consist only of low-Z elements, i.e., without any high-Z element probes. On the other hand, a structure labeled with a high-Z element probe is rather effectively visualized in an absorption contrast image in comparison with a phase contrast image. This is because the X-ray absorption is approximately proportional to the product of electron density and the cube of atomic number Z, while the X-ray phase shift is approximately proportional to only the electron density. From these considerations, we recommend absorption contrast microtomography as the primary method for visualizing high-Z element probes.

2.2. High-Z element probes

Element probes that have been reported for visualizing biological microstructures include osmium (Ananda et al., 2006; Johnson et al., 2006; Litzlbauer et al., 2006; Lareida et al., 2009; Kamenz and Weidemann, 2009; Mizutani et al., 2009b), gold (Mizutani et al., 2006, 2007, 2008b,c, 2009b, 2010b; Li et al., 2010; Nelson et al., 2010; Wang et al., 2011), silver (Mizutani et al., 2008a,b, 2010a; Parameswaran et al., 2009), iodine (de Crespigny et al., 2008; Metscher, 2009; Lee et al., 2010; Jeffery et al., 2011), platinum (Mizutani et al., 2008b, 2008c), mercury (Mizutani et al., 2009b), tungsten (Metscher, 2009), and lead (Kamenz and Weidemann, 2009). These elements, which are in the fifth or sixth row of the periodic table, can give sufficient contrast in an X-ray image represented with linear absorption coefficients (LACs). Elements with atomic numbers of 67–83, including osmium, gold, platinum, mercury, tungsten, and lead, have their L_{III} absorption edges between 8 keV and 14 keV, which falls within the range of hard X-ray energy typically available at synchrotron radiation facilities. In this section, we discuss the characteristics of each probe element and give a labeling method.

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