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Review

Imaging and characterizing cells using tomography

Myan Do [a,b,c], Samuel A. Isaacson [d], Gerry McDermott [a,b,c], Mark A. Le Gros [a,b,c], Carolyn A. Larabell [a,b,c,*]

[a] Department of Anatomy, University of California San Francisco, San Francisco, CA 94143, United States
[b] National Center for X-ray Tomography, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, United States
[c] Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, United States
[d] Department of Mathematics and Statistics, Boston University, Boston, MA 02215, United States

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ABSTRACT

We can learn much about cell function by imaging and quantifying sub-cellular structures, especially if this is done non-destructively without altering said structures. Soft X-ray tomography (SXT) is a high-resolution imaging technique for visualizing cells and their interior structure in 3D. A tomogram of the cell, reconstructed from a series of 2D projection images, can be easily segmented and analyzed. SXT has a very high specimen throughput compared to other high-resolution structure imaging modalities; for example, tomographic data for reconstructing an entire eukaryotic cell is acquired in a matter of minutes. SXT visualizes cells without the need for chemical fixation, dehydration, or staining of the specimen. As a result, the SXT reconstructions are close representations of cells in their native state. SXT is applicable to most cell types. The deep penetration of soft X-rays allows cells, even mammalian cells, to be imaged without being sectioned. Image contrast in SXT is generated by the differential attenuation soft X-ray illumination as it passes through the specimen. Accordingly, each voxel in the tomographic reconstruction has a measured linear absorption coefficient (LAC) value. LAC values are quantitative and give rise to each sub-cellular component having a characteristic LAC profile, allowing organelles to be identified and segmented from the milieu of other cell contents. In this chapter, we describe the fundamentals of SXT imaging and how this technique can answer real world questions in the study of the nucleus. We also describe the development of correlative methods for the localization of specific molecules in a SXT reconstruction. The combination of fluorescence and SXT data acquired from the same specimen produces composite 3D images, rich with detailed information on the inner workings of cells.

Introduction

Imaging is absolutely fundamental to continued progress in cell biology. While this may sound like a bit of an overstatement, it is difficult to deny the historic and ongoing contribution of microscopy to our understanding of cells [1]. Indeed, highly magnified images of cells are the context into which all other biochemical, biophysical and genetic measurements are viewed [2]. In an ideal world, data from a single microscope would be sufficient information to build a complete picture of a cell, but in reality this is an impossible dream [3]. Each type of microscope has unique imaging characteristics and, as a result, is only capable of generating data within a well-defined window of spatial resolution and image formation mechanisms [4,5]. To overcome this limitation a range of different, information-specific microscopes are used to image cells. For example, fluorescence microscopes are typically used to track and locate the positions of tagged molecules in the cell [6–9]. Electron microscopy, on the other hand, is best suited to generating very high spatial resolution images of the cellular ultrastructure [10,11]. The availability of new technologies – such as CCD[1] cameras, laser illumination sources, genetically encodable fluorescent labels, and high-performance computers – together with the implementation of new powerful, high-performance algorithms are pushing light- and electron-based microscopies to new limits. However, electron- and light-based methods are not well suited to imaging native-state specimens at the functionally important mesoscale (i.e. at a spatial resolution of a few tens of nanometers) [12,13].

* Corresponding author at: Department of Anatomy, University of California San Francisco, San Francisco, CA 94143, United States.
E-mail address: Carolyn.Larabell@ucsf.edu (C.A. Larabell).

[1] *Abbreviations used:* ALS, Advanced Light Source; CCD, charge-coupled device; CLM, cryogenic confocal light microscope; CFT, cryogenic fluorescence tomography; CFT–SXT, correlated CFT and SXT; FBP, filtered back projection; GFP, green fluorescent protein; LAC, linear absorption coefficient; NCXT, National Center for X-ray Tomography; OSN, olfactory sensory neurons; SXM, soft X-ray microscopy; SXT, soft X-ray tomography; Xi, inactive X chromosome; ZP, zone plate.

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The development of soft X-ray based methods for imaging cells filled this gap in cellular imaging [14].

Soft X-rays penetrate hydrated specimens more deeply than electrons do, and produce bright-field images with much higher resolution than is possible using light [15–17]. As a result, eukaryotic cells can be imaged intact, without sectioning or milling [18–21]. In soft X-ray microscopy (SXM) the image contrast is generated directly by the bio-molecular composition at each point in the cell [17,22]. There is, therefore, no need to stain cells, or treat them with heavy metals or other contrast enhancing agents prior to imaging [23]. Due to these advantages in specimen preparation, soft X-ray microscopy meets the criteria of complementing data from existing modalities while providing totally unique views of cells.

As with the majority of microscopes, SXMs only produce 2D projection images of the specimen [24]. The complexity of even a simple cell makes projection images difficult if not impossible to interpret, since sub-cellular structures end up confusingly superimposed in the image. To address this shortfall, tomographic methods are used to calculate 3D reconstructions of the specimen from a series of 2D SXM images [25]. Below we describe the application of transmission soft X-ray tomography (SXT) to imaging cells. We will also discuss the development of correlative methods that can partner with SXT, specifically the recent development of cryogenic fluorescence tomography (CFT).

In correlative CFT–SXT imaging the cryopreserved specimen is imaged in a cryogenic high-numerical aperture light microscope prior to being cryo-transferred to the SXM for acquisition of SXT data [26–29]. Landscape markers, clearly visible in data from both modalities, are used to accurately overlay one data set onto the other to produce a 3D multi-modal reconstruction [26]. In such analyses, labeled-molecules are localized precisely within high-resolution reconstructions of the cell. This combination of data from two complementary modalities produces significantly greater insights than is possible using SXT alone. We confidently predict this correlative method will become an important new tool for elucidating connections between molecular events and cellular phenotype. Correlated CFT–SXT can be applied to virtually any type of cell, presenting enormous imaging opportunities to the cell bio-research community. For this manuscript we will use the eukaryotic nucleus as a working example, since imaging and quantifying this organelle in its near-native state previously proved to be very challenging using other modalities [30]. Using CFT–SXT the nucleus can be fully segmented, based on the degree of chromatin compaction together with guidance from localized fluorescent molecules [31]. Information on the topology and packaging of the genome in the nucleus is invaluable to the generation of mathematical models that describe, for example, the movement of transcription factors and other molecules within the packed nuclear space [32]. Finally, the CFT data is used to unambiguously identify fluorescently labeled chromosome territories, creating a perfect structural complement to high-throughput molecular biology techniques, such as chromosome conformation capture (3C) [33].

Soft X-ray microscopy

If you have used a simple bright-field light microscope, you will already be familiar with the optical layout of a transmission soft X-ray microscope (SXM). In common with light microscopes, a SXM consists of a condenser lens, which focuses illuminating light onto the specimen, a specimen stage, and an objective lens, which focuses the light transmitted through the specimen onto a detector [15,16,34,35]. Typically, the detector is a thinned back-illuminated charge coupled detector (CCD) that directly measures photons transmitted by the specimen (as opposed to using a scintillator that is either bonded or fiber-coupled to the detector) [24]. Where light microscopes and SXMs differ most is in the detailed construction of the lenses. The refractive index of most materials towards soft X-rays is close to unity, making glass lenses unsuitable for use in SXM (besides, normal incidence glass lenses, such as those used in a typical light microscope, would completely attenuate the illumination). Instead, SXMs are equipped with either capillary optics and/or Fresnel zone plates (ZP) [16,36]. ZPs are diffractive optics, nanofabricated to generate concentric rings that alternate between opaque and transparent in the soft X-ray regime [37]. The maximum spatial resolution with which a SXM can image is generally determined by the thickness of the outermost ring in the objective lens [16,36,37]. The current generation of ZP objectives in everyday SXM use has a maximum resolution of 25 nm, but there are also ZPs available with an outer zone width approaching 10 nm [38]. These very high-resolution lenses could be fitted into existing SXMs easily, since spatial resolution has no impact on the physical size of the ZP. However, as the spatial resolution increases the depth of field (DOF) decreases [39]. The DOF for a 10 nm objective ZP is in the region of 500 nm to 2 μm, depending on the exact configuration of the instrument [34]. This DOF is smaller than most cells, certainly almost all eukaryotic cells. But, the limitations imposed by a shallow DOF could be overcome by collecting through-focus data at each angle in the series. Consequently, work is now underway to develop imaging acquisition and data processing strategies that permit use of enhanced resolution ZPs.

As with all microscopes SXMs require a source of illumination. In the case of a SXM this currently means a beam of soft X-ray photons produced by a synchrotron. Synchrotrons, such as the

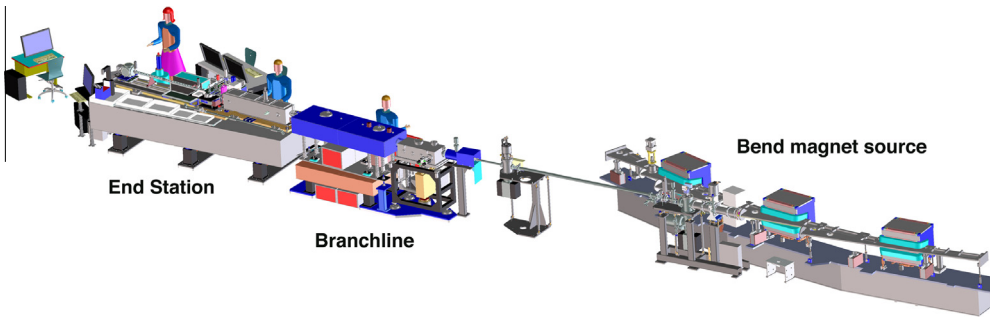

Fig. 1. Schematic of XM-2, a transmission soft X-ray microscope located at the Advanced Light Source, Berkeley. A bend-magnet in the synchrotron lattice generates an intense beam of radiation, which is transmitted to a flat plane mirror in the section of the instrument known as the branchline. The radiation then passes into the microscope room (this part of the instrument is termed *the end station* in synchrotron jargon). The condenser zone plate, in conjunction with a pinhole, acts as both a monochromator (to remove higher order radiation) and a focusing optic. The monochromatic illumination is focused to a 1×1 μm spot. To accommodate specimens larger than this spot size the condenser zone plate is scanned across the field of view during data collection. The specimen is mounted on a cryogenic rotation stage and cooled by a stream of cryogenic temperature gas at atmospheric pressure. Transmitted illumination from the specimen is focused onto a CCD detector by an objective zone plate.