Finding local order in cellular systems

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PERSPECTIVE

Finding local order in cellular systems

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Abstract

Specific local arrangements of molecules are the structural fingerprints of important biological processes in cells and tissues but difficult to access experimentally. In the recent work by Bernhardt *et al* (2017 *New J. Phys.* **19** 013012) such order on the nanometer scale has been investigated by *in situ* correlation of fluorescence-based cell visualization and nano-focused x-ray diffraction. This approach enables selective diffraction analysis guided by fluorescence imaging and opens new perspectives for the investigation of ordered nanostructures in living matter such as fiber bundles, membrane architectures, and newly-formed biominerals.

Main text

Molecular structures on the length scale of nanometers often govern biological function on much larger scales in the context of cells and tissues. A fundamental challenge in the fields of biophysics and structural biology is that local nanometric order is difficult to identify in heterogeneous cellular surroundings. The crucial molecular details such as functional protein or membrane arrangements are accessible immediately only when biological matter is organized homogeneously over macroscopic length scales. This is because one finds essentially the same molecular-scale structural features all over the sample. Already in the first half of the last century x-ray diffraction studies on macroscopic but spatially homogeneous biomaterials revealed relationships between nanometric structures and macroscopic functions. Examples are pioneering studies on the organization of collagen in rat tail tendon [2], on myelin sheaths around nerves [3], and on muscle structure and contraction mechanisms [4]. Notably, a muscle specimen was studied in the very first diffraction experiment exploiting synchrotron radiation [5]. It has become clear, though, that the situation is more difficult for the vast majority of molecular-scale structures in cells and tissues. While a high degree of organization in terms of periodic arrangements and molecular orientations can be found locally on length scales of hundreds of nanometers [6], the order is lost, when structural information is averaged over larger volumes.

A commonly taken approach to overcome this problem is to use high resolution techniques in scanning mode to characterize the entire volume of biological samples with the aim to come across regions with the molecular-scale structural features of interest. To this end, scanning electron microscopy with focused ion beam milling (Cryo FIB-SEM) [7] and imaging x-ray diffraction [8] are nowadays well-established. In the latter case, micron- or sub-micron sized x-ray beams are moved across the sample, so that at each scanning point a diffraction pattern containing the information on molecular-scale structures is obtained [9]. Such scanning approaches, however, imply a number of limitations. At first, the scanning process is typically too slow to obtain images of living biological specimens. Secondly, the sample fixation needed for scanning is usually invasive and sample damages can be substantial. Finally, most of a data set is useless when entire sample volumes are scanned while features are only locally of interest. A fascinating advancement is correlative imaging, where an imaging technique of moderate resolution such as optical microscopy or x-ray micro-computed tomography (μCT) is used to cover large sample volumes and to identify relevant spots for a localized high-resolution structural probe. This approach, which is fast and minimizes the overall radiation damage to the sample, has been applied successfully to hierarchical structures in biological materials, e.g. to map mineral particle characteristics during the course of healing in rat bone by combining electron microscopy, μCT and scanning x-ray diffraction [10].

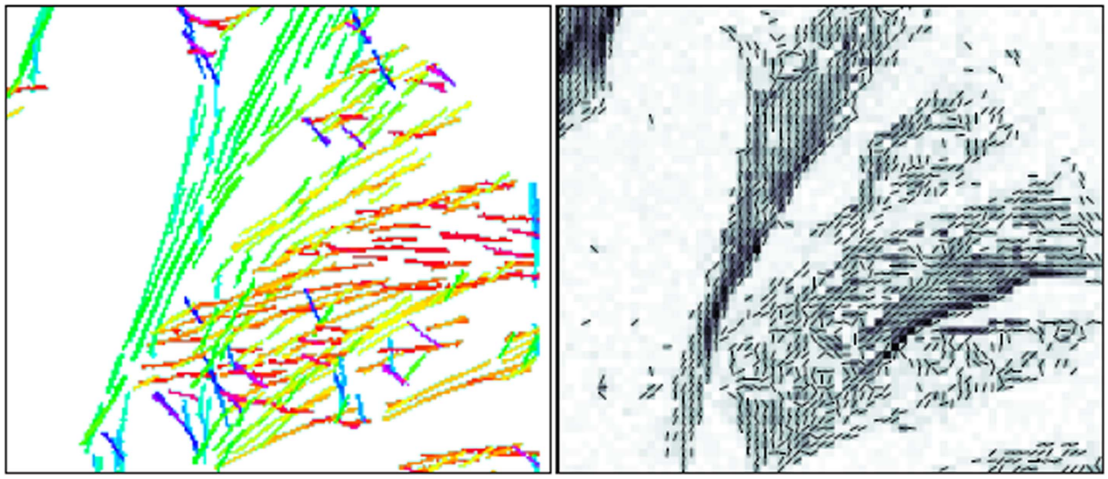

Figure 1. Correlative imaging of the cytoskeleton network in neonatal cardiac tissue cells. (left) Most significant actin fibers as identified by optical fluorescence microscopy. Colors indicate orientation angles. (right) Orientation angles as obtained from the x-diffraction anisotropy, indicated as black lines. Figure adapted from Bernhardt *et al* [1].

One of the most successful correlation approaches in bioscience is the combination of optical fluorescence and 3D electron microscopy with high sensitivity and spatial precision [11]. In-situ scanning x-ray diffraction in combination with light microscopy has recently been applied even to living cells in microfluidic environments to determine local structural information [12].

Bernhardt *et al* [1] have now demonstrated the feasibility of *in situ* correlative imaging between optical fluorescence microscopy and nano-focused x-ray diffraction at the sub-cellular level. Fluorescent labeling technologies are uniquely suited to study dynamic processes in living cells [13]. In the study by Bernhardt *et al* on actin-based cytoskeleton networks in neonatal cardiac tissue cells from rats the actin was fluorescently labeled and the fiber orientation in terms of direction and degree of orientation was quantified in each volume element from the local x-ray scattering anisotropy. The results showed excellent point-by-point match with the corresponding orientation maps in the fluorescence images (see figure 1). While most of the presented results were obtained with freeze-dried cells, experimental methodology and sample environments for recordings on (initially) alive cells were also established yielding promising first results. Their study lays the ground for the correlative determination of structures that are ordered on the nanometer scale and therefore only accessible by x-ray diffraction. More importantly, the *in situ* methodology established by Bernhardt *et al* allows performing x-ray diffraction selectively at the spots that according to the fluorescence image are likely to contain the nanometric structural features relevant for the observed cell function or behavior. Loss of structural information due to radiation damage, in turn, can be minimized, especially with the upcoming highly brilliant, pulsed x-ray sources. Together with fast online x-ray scattering data analysis during the experiment, *in situ* correlative determination of molecular-scale structures in living cells and tissues becomes possible. Such approaches will be most rewarding when applied to molecular arrangements with a high degree of structural ordering and when the molecular ordering exhibits the signatures of the particular biological function under investigation. Important examples include fiber bundles under tension or exposed to osmotic dehydration [14], naturally occurring membrane stacks during formation and maturation [15], or early stages in the formation of mineralized structures [16]. Ultimately, knowledge of local order may yield valuable insights into living matter.

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