New and Notable

Light Microscopy in Biological Research

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Optical microscopy and particularly fluorescence confocal microscopy is used on a large scale in the studies of cell structure and function including nuclear arrangement of chromatin. Chromosome territories and subchromosomal domains in cell nuclei as well as other nuclear structures are systematically investigated by means of three-dimensional fluorescence in situ hybridization and immunostaining in different stages of the cell cycle. Dynamics of structural changes can be investigated using green fluorescent protein conjugated to any other protein in live cell experiments. In these studies, high speed in parallel with resolution are important characteristics of the microscopic system. Recent development of confocal heads with improved light throughput and reduced bleaching and phototoxicity, powerful lasers, and back-illuminated charge-coupled device cameras with on-chip amplification, together with optimized computer control, fulfill to a large extent the requirements of recent research providing excellent tools for in situ proteomics.

The mentioned technological advances suffer from limited resolution given by the Abbe limit (~200 nm), which imposes undesirable limitations on research activities. It can be supposed and the latest data actually show that gene regulation is related to changes of chromatin structure on the level of individual genes (Chambeyron and Bickmore, 2004) with dimensions beyond the Abbe limit. There are several solutions that overcome the Abbe limit of optical microscopy (scanning probe techniques or electron microscopy), however, they are inappropriate for structural studies of three-dimensional objects and cannot be used for in vivo imaging. The resolution requirements are fulfilled by spatially modulated illumination microscopy, a relatively novel technique, based on periodic excitation along the axial direction (Hildenbrand et al., 2005), which gives rise to a similarly periodic emission profile from a fluorescent object placed between the two objectives. The object causes variation of the emission profile in a size-dependent manner. The ratio between the maximum of this variation and the maximum emission intensity gives the ''modulation contrast'' (R), which is directly proportional to the object size (e.g., 30–120 nm for 488-nm excitation).

The authors (Hildenbrand et al., 2005) demonstrated the feasibility of the measurements in fixed cells stained using fluorescence in situ hybridization, however, the spatially modulated illumination microscopy can be obviously applied to detect structures visualized by immunostaining. Further applications in both research and diagnostics can be expected. The importance of the new kind of microscopy can be documented by the fact that Leica Microsystems (Wetzlar, Germany) has recently introduced the so-called 4pi microscope system (Hell and Stelzer, 1992) that also uses wave fronts of two opposite objective lenses. The resolution in the z-direction is increased by a factor of 3–7. This new system allows imaging at or below 50-nm resolution.

It becomes clear that progress in biological research in the postgenomic era will depend on new technologies that provide information on epigenetic mechanisms regulating gene expression. In particular, physicochemical states of chromatin play a very important role in gene transcription (Cremer and Cremer, 2001). To understand regulatory functions of chromatin it is necessary to analyze higher-order chromatin condensation and nuclear organization. In other words, gene regulation is a highly complex, spatiotemporal biochemical process that strongly depends on nuclear structures, particularly on the structure of chromatin. To predict gene expression quantitatively, detailed information on structural characteristics of the cell nucleus is needed. Owing to its descriptive nature that does not explain mechanisms, structural biology has been for quite a long time in the shadow of other biological disciplines such as biochemistry, genetics, or molecular biology. At present, structural studies are urgently needed to overcome the evident barrier on our way to understand the quantitative nature of biological processes. The article of Hildenbrand et al. (2005) in this issue provides evidence that recent progress in optical microscopy meets the needs of biological research.

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