

CORRELATIVE MICROSCOPY: MULTIPLE DATA IMAGING BY LIGHT- AND ELECTRON MICROSCOPY (TUTORAL)

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INTRODUCTION: Imaging cells by light- or electron microscopy is based on entirely different preparation techniques and thus yield results with different qualities. A comparison of the high resolution of EM-images with multi-parameter imaging methods of modern light microscopy provides a key to unlocking structure-function relationships of cellular compartments and components. It also provides perhaps the best method for understanding the true nature of light-microscopic images. We describe here a working-strategy enabling the correlative analysis of typical parameters (x,y,z,t,λ) generated by digital wide field or confocal light microscopy.

Light microscopy permits observations under physiological conditions. This enables the comparison between bright field images (e.g. video-enhanced differential interference phase contrast (1) with fluorescence (e.g. auto-fluorescent proteins such as green fluorescence protein). Time-lapse observations can be supplemented with multi-immunofluorescence labelling on fixed cells; a final structural identification of the various light microscopic signals by electron microscopy culminates the many steps which are included in a exhaustive microscopic survey of a selected area of an object.

METHODS: The effort to achieve complete sampling of structural data on the same cell or subcellular region requires a sequence of preparatory and analytical steps, the most essential of which are summarized as follows:

1. In order to track the same cell through all the steps of microscopy, we have grown sub confluent monolayers on gridded coverslips (Cellocate, Eppendorf).
2. Observation of live cells under physiological conditions requires a microscope with a heated stage and a controlled atmosphere incubation chamber.
3. Time-lapse observations can be automated wide-field (1) or confocal (2) microscopes in various modes (x,y,t to $x,y,z,\lambda t$)
4. Fixation of cells (typically 3% paraformaldehyde followed by saponin for permeabilisation)
5. Immunoreactions with antibodies and fluorochrome conjugates and detection of multiple fluorescence signals by wide-field or confocal microscopy.
6. Postfixation with glutaraldehyde and osmium tetroxide, dehydration and embedding

for thin-sectioning and transmission electron microscopy. 7. Correlation of structures revealed by light microscopy in live-cells and/or fixed cells with that seen by transmission electron microscopy (TEM).

RESULTS: We applied the above protocol to study cells infected with herpes simplex virus (HSV). Light microscopy of living cells demonstrated the dynamics of virus-induced cytopathic effects and phenomena associated with the HSV assembly and egress from host cells. After fixation, the viral nature of vesicular structures was further characterized by multispectral immunofluorescence and 3D-confocal microscopy employing antibodies directed against various viral epitopes. Colocalisation of the several viral elements with cellular structures illustrated the various stages of viral maturation along the secretory pathway. Finally, TEM revealed the presence of viral particles and their association with the cellular compartments associated with the final stages of viral maturation.

DISCUSSION & CONCLUSIONS: Light microscopy generates a wealth of information based on the parameters x,y,z,t and λ . The images can be further enhanced by powerful 2D- and 3D-image processing and reconstruction techniques. The limits of resolution, however, often limit understanding the nature of the structures under study without precisely correlating light and EM images (3). In the system describe here, multidimensional microscopy allowed to study and characterize the interaction of sub microscopical virus particles with cellular substructures.

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