for live-cell imaging. However, performance of the technique is context-dependent: e.g., weak fluorescence signals and clustered sub-resolution structures typically yield poor deconvolution results. We have evaluated such difficulty using realistically simulated TIRF images of GLUT4 glucose transporters in cultured adipocytes, whose average diameter of 75nm is far below the optical resolution. An essential image-processing step isolating regions of high information content from a TIRF image was discovered, which enables subsequent deconvolution resolution approaching 100nm. Detailed analysis of deconvolution results as a function of signal-to-noise qualities of the original images suggests that super-resolution details can be resolved with TIRF images of live cells acquired at speed up to 10 fps.

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Trimming the resolution gap in the study of molecular and cellular events by means of High Data Output and automated three-dimensional Correlative Light-Electron Microscopy approach

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Correlative light/electron microscopy (CLEM) allows the simultaneous observation of a given subcellular structure by fluorescence light microscopy (FLM) and electron microscopy. The use of this approach is becoming increasingly frequent in molecular and cellular biophysics. Here we report on a new high data output three-dimensional (3D) CLEM method based on the use of cryosections (Vicidomini et al.,Traffic, 2008). We successfully applied the method to analyze the structure of rough and smooth Russell bodies used as model systems. The major advantages of this approach are the following: (i) the ability to correlate several hundreds of events at the same time, (ii) the possibility to perform 3D correlation, (iii) the potential to immunolabel both endogenous and recombinantly expressed proteins at the same time and (iv) the effective combination of the high data analysis capability of FLM with the high precision-accuracy of transmission electron microscopy in a CLEM hybrid morphometry analysis. We have identified and optimized critical steps in sample preparation, defined routines for sample analysis and retracing of regions of interest, developed software for semi/fully automatic 3D reconstruction and defined preliminary conditions for an hybrid light/electron microscopy morphometry approach. The relevance of the presented approach is further enhanced by two important key elements, namely: the development of optical nanoscopy methods and the potentiality for exploring different correlative frameworks like optical nanoscopy vs. optical microscopy adding scanning force microscopy techniques.

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Seeing Multifunctional Nano- and Micro-particles Suitable for Imaging & Therapy Using Freeze-fracture Electron Microscopy

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The potency of nano- and micro-particles, loaded with therapeutic and/or diagnostics, is frequently depending upon their morphology adopted in a biological relevant environment. Freeze-fracture electron microscopy (ff-em) as a cryofixation, replica TEM method is a powerful technique to monitor self-assembling of lipid-, polymer-, as well as protein/peptide-based carriers encapsulating drug-, gene-, vaccine, and imaging molecules[1-3]. At a resolution limit of 2 nm we are able to study the fate of such carriers related to their pay load, application milieu [4], and during their interaction with cells.
Using ff-em we studied the morphology of a wide variety of nano- and micro particles suitable as carriers for diagnostics as well as therapeutics including quantum dots (free and coupled to drug-loaded immunoliposomes), micelles (spherical-, disc-, and worm-type micelles) [5], small unilamellar liposome [6], multilamellar liposome, niosomes [7], cationic liposome/DNA complexes [8,9], polymer- or lipid-stabilized gas bubbles [10], cochleate cylinder, depofoam particles, and drug crystals. Recently we explored liposome-, virosome-, and virus-based vaccines, including measles vaccine powders, by ff-em.
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New Approach To Quantitative Subcellular Imaging Of Phosphorus And Calcium Using Energy-filtered Transmission Electron Microscopy And Tomography

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Analytical electron microscopy provides high-resolution distributions of chemical elements by measuring characteristic core-edge signals that originate from interaction of the incident electrons with a thin section of a cell. Such elemental images give information about the organization of specific biomolecules within cellular organelles, as well as the distribution of ions involved in regulation of cellular processes. For example, mapping phosphorus enables visualization of nucleic acid, and calcium provides distributions of a major intracellular second messenger. Elemental mapping at ~10 nm spatial resolution is typically achieved using energy-dispersive x-ray spectroscopy (EDXS) or electron energy loss spectroscopy (EELS) in a scanning transmission electron microscope (STEM). We have developed a complementary approach based on energy-filtered transmission electron microscopy (EFTEM). It is demonstrated that quantitative 2D elemental distributions containing ~10^6 pixels can be obtained from large regions of cells, and that 3D elemental distributions can be obtained when EFTEM is combined with electron tomography. It is found that an accurate elemental distribution can be derived from just two energy-selected images, above and below a core-edge. However, since the core-edge signals for elements like calcium and phosphorus are relatively weak, it is important to model the spectral background carefully by correcting for plural scattering. We have applied quantitative ETFEM imaging and tomography to determine the 3D distributions of DNA in the cell nucleus, and to measure calcium in mitochondria of neurons.

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Investigating the Protective Effects of Milk Phospholipids Against Ultraviolet Exposure Using Confocal Reflectance Microscopy

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Current research on bioactive molecules in milk have documented health advantages of bovine milk and its components. Milk phospholipids, selected for this study, represent molecules with great potential benefit in human health and nutrition. In this study we used confocal reflectance microscopy to monitor changes in skin morphology upon exposure to ultraviolet light and evaluate the potential of milk phospholipids in preventing photodamage to skin. We imaged skin equivalent models based on human keratinocytes and dermal fibroblasts cultured in a collagen matrix. We compared images from skin equivalent models with (a) no exposure to UV light, (b) exposure to a dose of 60 mJ/cm^2 of UVB exposure, triple the minimal erythema dose, (c) exposure to milk phospholipids in the media, and (d) exposure to milk phospholipids in the media followed by exposure to UV light. Specimens were imaged directly after exposure, 24 hours after exposure, and 48 hours after exposure. The results suggest that milk phospholipids act upon skin cells in a protective manner against the effect of ultraviolet radiation. Preliminary experiments determining the mechanisms by which the benefits occur are underway.

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Biomineralization By The Marine Tubeworm Hydroides Dianthus: Structure And Composition Of The Adhesive Cement

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The structure and composition of the adhesive cement of Hydroides Dianthus was studied using a variety of characterization techniques, including XRD, FTIR, SEM, EDX, and AFM. The cement was determined to be a composite of inorganic CaCO3 crystals in an organic matrix, with the organic component making up only a small fraction of the material. Two polymorphs of CaCO3, in roughly equal proportions, were identified in both the tube shell and the cement via XRD and FTIR: aragonite (CaCO3), and magnesium calcite ((Ca,Mg)CO3). Electron microprobe and EDX measurements also confirmed the presence of magnesium. SEM imaging revealed two distinct crystal habits, and EDX measurements allowed for the identification of crystals with an acicular habit as aragonite, and crystals exhibiting a triangular layered structure as magnesium calcite. AFM measurements in sea water and in air were performed in order to determine the elastic moduli of the various components of the composite cement. For the inorganic component, moduli in the range of ~3 GPa were observed in the wet state, and values in the range of ~11 GPa were