In focus in HCB

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The quantitative morphological 3D analysis of organelles of highly complex structure such as the Golgi apparatus is laborious and time consuming, but volume scanning electron microscopy (volume-SEM) methods have the potential to overcome this problem through automated sectioning and imaging. In their Review, Ferguson et al. (2017) provide a comprehensive and critical analysis of various volume-SEM techniques. As they point out, volume-SEM as applied to the study of the Golgi apparatus also produces large data sets, and subsequent quantitative 3D reconstruction is labor-intensive. Hence, the challenge is to optimize the processing of large amounts of 3D information. As Ferguson et al. (2017) emphasize, this can be achieved using a sampling-based stereological approach that reduces the amount of work without compromising precision and providing minimally biased estimates. The stereological approach consists of conventional random sampling, or its modification, which is called systematic uniform random sampling, and the application of estimators for sensing 3D quantities (volume, surface, length or number) from 2D profiles in the EM images. The authors highlight the importance of one factor which is central for any type of quantitative estimation in EM: the rigorous application of criteria for organelle or structure identification.

Tissue autofluorescence is oftentimes a bane to specific antibody recognition in fluorescence and confocal microscopy. Many methods and protocols have been developed

and offered to combat the multiple types of autofluorescence present in various cells and tissues. A sampling of some of these methods includes: (1) irradiation of the tissue section with artificial visible light (Neumann and Gabel 2002) or filtered sunlight (Kingsley et al. 2001); (2) treatment with copper sulfate, ammonia–ethanol, sodium borohydride or Sudan Black (Schnell et al. 1999; Baschong et al. 2001); (3) elimination by digital image processing (Van der Lest et al. 1995); (4) employing far red fluorophores (Pier et al. 2011); (5) utilizing customized filter sets on the microscope (Pang et al. 2013); (6) multispectral imaging followed by linear unmixing (Mansfield et al. 2008). It is this last approach for dealing with tissue autofluorescence that Peter et al. (2017) employed for detecting specific immunostaining on sections from human lung, a tissue with well-documented autofluorescence issues. These authors were interested in the potential effect of pneumococcal infection on barrier function and junctional proteins in the alveolar-capillary space. They used an ex vivo model of human lung tissue to analyze the expression of multiple junctional proteins in control and *Streptococcus pneumoniae* (*S. pneumoniae*)-infected tissues. Employing spectral imaging confocal microscopy, followed by a linear unmixing algorithm, they were able to successfully remove background autofluorescence from specific antibody-based fluorescence signal. Their experiments demonstrated that infection with *S. pneumoniae* resulted in a diminution of alveolar-localized occludin, ZO-1, claudin-5, and VE-cadherin, while levels of claudin-3, -4, and -18 appeared to be unaffected, results which were corroborated by western blot analysis of these proteins. Interestingly, claudin-3, -4, and -18 were expressed exclusively in alveolar type II epithelial cells, and their expressions were not affected by *S. pneumoniae*, prompting the authors to propose further studies to investigate this finding. This manuscript highlights

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the clever use of confocal spectral imaging combined with linear unmixing to remove autofluorescence signals from an image rather than relying on sometimes unpredictable tissue section chemical or light wave exposure.

Lopez-Fontana et al. (2017) investigated the molecular mechanisms behind a potential link between thyroid-related conditions and breast cancer. In an earlier manuscript, these same authors (Lopez-Fontana et al. 2013) had shown that hypothyroidism resulted in the following effects on mammary tumor formation: (1) prolonged time until tumor appearance; (2) reduction in tumor occurrence; (3) retarded tumor growth; (4) increased rate of apoptosis. In the current manuscript, they investigated a possible interaction between thyroid hormones and the Wnt/ β -catenin pathway in breast cancer, since a relationship between them has been established in other tissues. Using a rat model of mammary tumor formation induced by treatment with 7,12-dimethylbenz(a)anthracene (DMBA) in hypothyroid and euthyroid animals, they studied the expression and localization of β -catenin with apoptosis. Using the techniques of immunohistochemistry and western blot analysis they found that the expression of Bax, cleaved caspase-9, and caspase-3 was significantly elevated in the tumors of hypothyroid compared to euthyroid animals. Moreover, β -catenin was localized to the plasma membrane, and in decreased intensity in hypothyroid animals, whereas it was restricted to the nucleus and at greater intensity in euthyroid animals. These results suggest that hypothyroidism is associated with activation of the intrinsic apoptotic pathway in rat mammary tumors affecting tumor growth and progression.

The apoE/LDLR^{-/-} mice spontaneously develop atherosclerotic plaques, and represent an animal model to analyze the changes of cellular and extracellular components during development and progression of atherosclerosis. As an alternative to the commonly used oil red O staining of frozen sections to quantify the atherosclerotic lesions in apoE/LDLR^{-/-} mice, Gajda et al. (2017) report a polychrome histochemical staining that permits the simultaneous identification of various plaque components in sections of formalin-fixed and paraffin-embedded atherosclerotic arteries. The authors combined Martius yellow–crystal scarlet–methyl blue (MSB) to stain fibrin, erythrocytes and collagen fibers, with orcein to stain elastic fibers. The OMSB staining applied to sections of brachiocephalic arteries permitted the multicolor discrimination of the various plaque components such as collagen, elastic fibers, fatty streaks, fibrin, foam cells and intraplaque erythrocytes. Importantly, the OMSB histochemical staining method can be used with automatic stainers. Most important is the finding that the good contrast and sufficient color separation obtained with

the OMSB staining permits digital automatic segmentation of the various plaque components and, therefore, the automated quantitative plaque analysis. Together, the OMSB polychrome staining represents a most useful routine tool for the qualitative and quantitative assessment of atherosclerotic lesions.

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