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Invited Review

Exploring the third dimension: Volume electron microscopy comes of age☆

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ABSTRACT

Groundbreaking advances in volume electron microscopy and specimen preparation are enabling the 3-dimensional visualisation of specimens with unprecedented detail, and driving a gratifying resurgence of interest in the ultrastructural examination of cellular systems. Serial section techniques, previously the domain of specialists, are becoming increasingly automated with the development of systems such as the automatic tape-collecting ultramicrotome, and serial blockface and focused ion beam scanning electron microscopes. These changes are rapidly broadening the scope of biomedical studies to which volume electron microscopy techniques can be applied beyond the brain. Further innovations in microscope design are also in the pipeline, which have the potential to enhance the speed and quality of data collection. The recent introduction of integrated light and electron microscopy systems will revolutionise correlative light and volume electron microscopy studies, by enabling the sequential collection of data from light and electron imaging modalities without intermediate specimen manipulation. In doing so, the acquisition of comprehensive functional information and direct correlation with ultrastructural details within a 3-dimensional reference space will become routine. The prospects for volume electron microscopy are therefore bright, and the stage is set for a challenging and exciting future.

Contents

1. Introduction

It has been written that electron microscopy (EM) is currently undergoing a revival, with a resurgence of interest in this informative yet complex ultrastructural analysis technique (Krijnse Locker and Schmid, 2013; Kuwajima et al., 2013a; McDonald and Auer, 2006). Despite provocative titles that suggest otherwise (Knott and Genoud, 2013), EM specialists would agree that their own enthusiasm and workload remains undiminished. Indeed, novel technologies are emerging apace, alongside advances in specimen

Abbreviations: ATUM, automatic tape-collecting ultramicrotome; ATLUM, automatic tape-collecting lathe ultramicrotome; CLEM, correlative light and electron microscopy; CLVEM, correlative light and volume electron microscopy; EM, electron microscopy; ER, endoplasmic reticulum; ET, electron tomography; FIB SEM, focused ion beam scanning electron microscopy; TEM, transmission electron microscope; ssTEM, serial section transmission electron microscopy; SEM, scanning electron microscope; SBF SEM, serial blockface scanning electron microscopy; ssSEM, serial section scanning electron microscopy.

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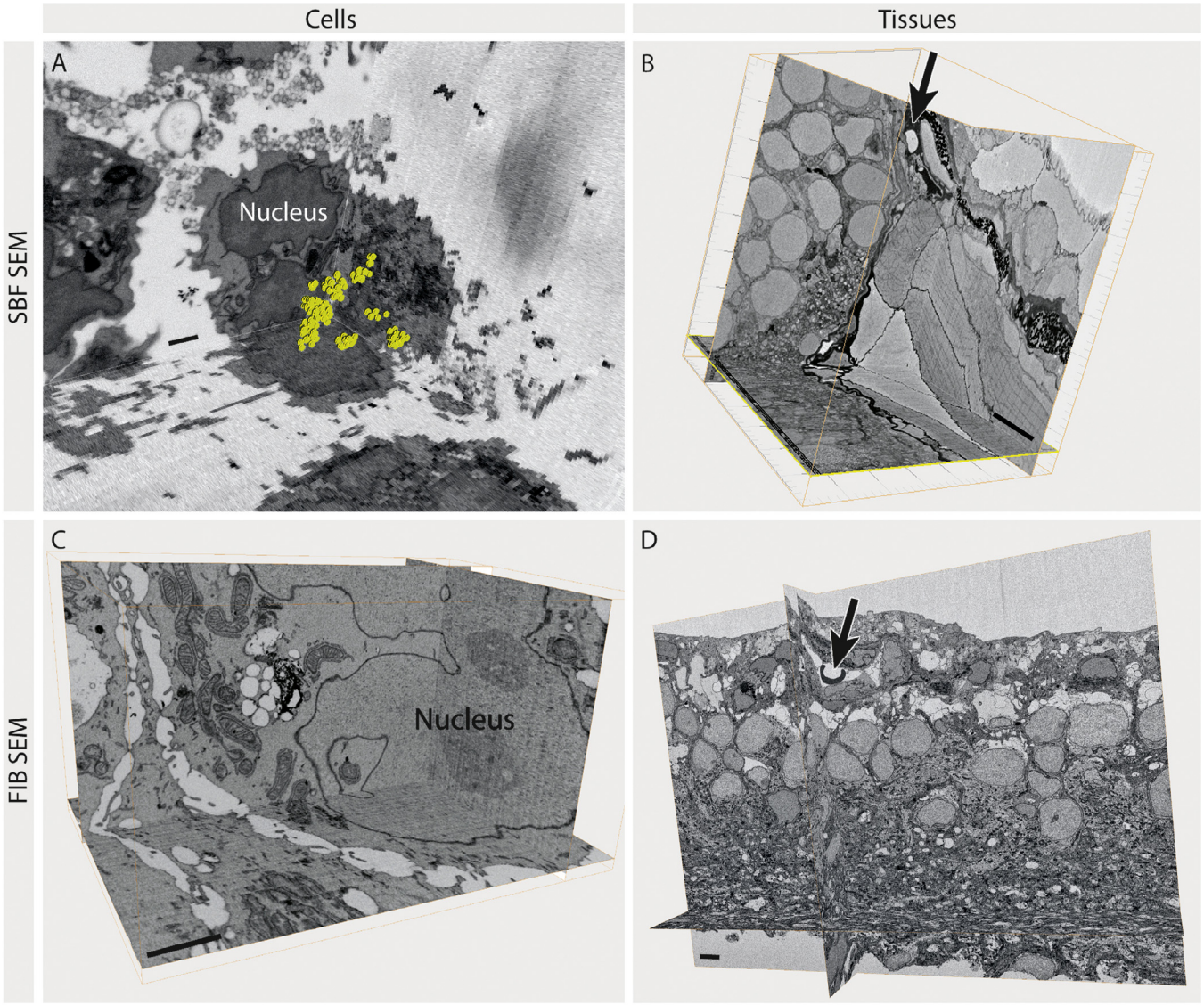

Fig. 1. Examples of volume EM data from 'non-brain' specimens. Serial Block Face SEM data showing (A) B cells containing 200 nm beads (Thaunat et al., 2012) and (B) zebrafish embryo blood vessels (arrow; Armer et al., 2009). Focused Ion Beam SEM data showing (C) fibroblasts and cancer cells in a 3D matrix (L. Collinson, E. Sahai and A. Schertel) and (D) mouse retinal blood vessels (arrow; Stenzel et al., 2011). Scale bars are estimates as the images are 2D projections of 3D data. Bars: *A*, *B* ≈ 1 μm; *C*, *D* ≈ 5 μm.

preparation techniques and significant developments in instrumentation.

One area of rapid development is volume electron microscopy (volume EM), a collective term for EM techniques focusing on analysis of 'large' volumes ('large' being a relative description in the EM world). Most techniques that fall under the volume EM umbrella were initially developed for examination of the central nervous system (Denk and Horstmann, 2004; Knott et al., 2008; Micheva et al., 2010; Micheva and Smith, 2007), a result of the need to analyse axons and dendrites that span large distances (10^{-1}–10^{-5} m) with sufficient resolution to detect individual synaptic vesicles and densities (10^{-9} m). The associated technical challenge lay in overcoming the 'field of view versus resolution' problem, to enable visualisation of a single specimen across different scales, a problem common to most imaging modalities. The use of volume EM in neuroscience has been comprehensively reviewed (Arenkiel and Ehlers, 2009; Briggman and Bock, 2012; Denk et al., 2012; Eisenstein, 2009; Helmstaedter, 2013; Helmstaedter et al., 2008; Kleinfeld et al., 2011; Smith, 2007). Here, we focus on the development and application of these emerging techniques and technologies to biomedical specimens beyond the brain (examples shown in Fig. 1).

Volume EM can be performed using transmission or scanning electron microscopes. Each approach has its own strengths and weaknesses, and the choice is dependant on the required lateral (*x*, *y*) and axial (*z*) resolution, and the size of the structure of interest. Historically, transmission electron microscopy (TEM) was the tool of choice for ultrastructural examination of biomedical specimens at sub-nanometer resolution. However, for many cell biology studies structural resolution is actually limited by the deposition of heavy metals onto membranes during sample preparation. In addition, voxel dimensions may only need to be half that of the smallest expected feature of interest (Briggman and Bock, 2012). Advances in scanning electron microscopy (SEM) technology are now driving a paradigm shift in electron imaging. SEMs with field emission electron sources and high efficiency electron detectors can achieve lateral resolutions in the order of 3 nm, allowing visualisation of structures such as synaptic vesicles and membranes (De Winter et al., 2009; Knott et al., 2008; Vihinen et al., 2013; Villinger et al., 2012), though resolving individual leaflets of membrane