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Building a Research Vision: A Visible Cell takes shape

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In the 25 years since Dubochet and McDowall's vitrification of water for cryo-electron microscopy (cryo-EM) [1], much has been achieved in the investigation of biological ultrastructure at low temperatures. However, much still remains to be done - and seen. Cryo-EM studies of thin layers of vitrified aqueous suspensions have elegantly shown biological macromolecules in a near-native state at high resolution and when combined with tomographic methods for 3D reconstruction, cryo-EM represents an important tool in molecular biology. One solution for investigating the ultrastructure of cells or tissues in a near-native state resides in **cryo-EM of vitreous sections (CEMOVIS).** Fortunately, technology here has also progressed and obstacles to cryo-section production are gradually being overcome. Considered the "dream method" by many structural cell biologists, CEMOVIS involves the vitrification of cells or tissue by rapid freezing, followed by sectioning and observation of the frozen-hydrated material at low temperature [2,3,4]. The full potential of this method can be realized when it is combined with cryo-EM tomography for 3D reconstruction and analysis of cell or tissue ultrastructure at high resolution.

To address such initiatives in molecular cell biology, the Centre for Microscopy & Microanalysis (CMM) at the University of Queensland (UQ) and the Nanostructural Analysis Network Organisation (NANO) have established world-class core microscopy and microanalytical resources that will be critical for the success and development of successful research programs. The UQ node of the NANO - Major National Research Facility (MNRF) is run through the CMM, which manages 15 major microanalytical research instruments on campus. As the foundation Queensland node of the NANO-MNRF, the purchase of a 300 keV Tecnai F30 transmission EM equipped with two ultrahigh sensitivity UltraScan CCD cameras and a Gatan Imaging Filter (GIF) is the culmination of many months of strategic planning involving Federal and State funding bodies, Australian Universities and International vendors. However, the NANO-MNRF facility at UQ represents more than just the purchase of new instrumentation. It embodies the marriage of infrastructure and technologies available through all participating national microscopy and research institutions in Australia. The successful purchase and access to advanced instrumentation and technologies through an interconnected national microanalysis **network** is a unique venture for the region. Table 1.

Examples of research program goals that are underpinned by CMM/NANO facilities include the Visible **Cell Project**, which aims to produce visually-interactive representations of living cells that approach macromolecular resolution, using a unique combination of advanced structural biology, proteomics, informatics, computational and visualization approaches and expertise; **Cell Architecture and Trafficking**, which focuses on the study of macromolecular organization and dynamics; and the **Virtual Membrane Project**, which sets out to create a micro-compartmentalized plasma membrane in silico for real-time analysis of spatially-segregated signalling cascades.

Collectively, these programs have already received considerable support from the NANO-MNRF, the Australian Cancer Research Foundation and the Australian Research Council (ARC), and represent major initiatives of the Institute for Molecular Bioscience (IMB) and the University of Queensland through significant infrastructure support for the integration of cryo-EM, EM tomography, advanced 3D visualization and high-performance computing. Studies currently underway include cryo-EM tomography of vitrified sections cut from *Chlamydomonas rheinhardtii*, which is expected to provide new insights into the 3D organisation of the light-capturing machinery of Photosystem II, and may yield valuable information on the functional interaction that occurs between mitochondria and chloroplasts (Figure 1).

References

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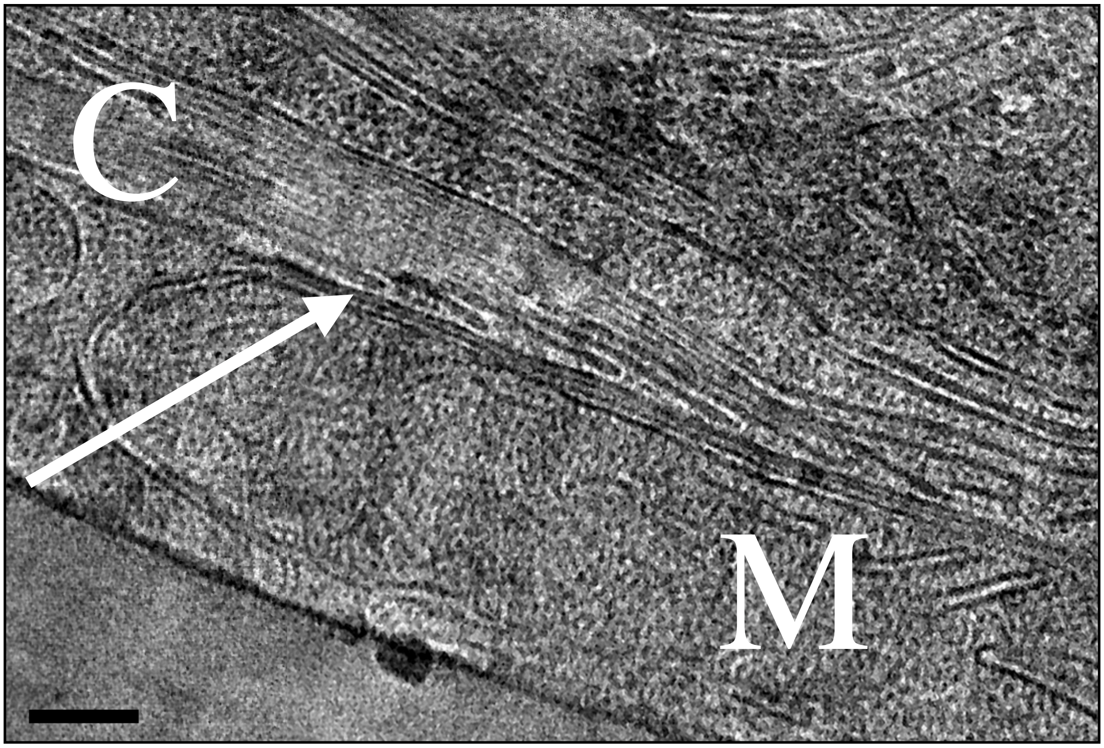

FIG. 1.
A frozen-hydrated section cut from high-pressure frozen *C. rheinhardtii* (green algae), imaged at -178°C on a Tecnai TEM. The micrograph reveals chloroplast grana membrane stacks (C), at the mitochondrial membrane (M) interface (arrow). Scale bar, 0.3 μm.

TABLE 1. NANO core instrumentation, shaded section describes the Queensland microscope node.

INSTRUMENT	NANO NODE LOCATION	APPLICATIONS
300keV FEG Transmission Cryo-Electron Microscope	**Centre for Microscopy and Microanalysis at Queensland Bioscience Precinct (The University of Queensland)**	**Cell tomography. Single particle macromolecular imaging. Protein crystallography 3-D imaging of nano-structured materials.**
Advanced Atom Probe Platform	Australian Key Centre for Microscopy and Microanalysis (University of Sydney)	Atomic resolution imaging. Atomic scale manipulation. Nanoscale fabrication.
Advanced Focussed Ion Beam Platform	Electron Microscope Unit (University of New South Wales)	Multi-source milling. Single atom implantation.
Nano-Secondary Ion Mass Spectrometry, NanoSIMS	W .A. Centre for Microscopy (University of Western Australia)	Nanoscale geochronology. Nanoscale mapping. Sub-cellular isotope tracer mapping.

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