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Creating next-generation microscopists: structural and molecular biology at the crossroads

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Abstract

This paper highlights the importance of advanced microscopy and microanalysis in the pursuit of quality research in the biological and life sciences. With the growing complexity of modern microscopes, there is substantial risk of incorrect use or misinterpretation of data by the inexperienced researcher. This paper emphasizes the need for collaboration between biological microscopists and molecular biologists, within the context of centralized facilities and supported by first-class training, to fully realize the power of these unique instruments in modern biology and to create the next generation of molecular microscopists.

Keywords: centralized microscopy facilities • correlative imaging • molecular microscopy • sample preparation

Modern biomolecular microscopy

Gone are the days when the typical morphologist sought to understand the complex interior of the cell with his 'magnifying glass' while his colleague, possibly even in the same institution, pursued the same quest with advanced molecular biology techniques. Instead, structural biology and molecular biology are closely integrated in the modern research environment thanks to the development of novel imaging biomolecular microscopy platforms [1], which have arisen from new, powerful computers, from fast high-resolution cameras, from improved image processing software, and from advances in various molecular probes specifically designed for labelling fixed and/or living cells [2]. The current boom in the development of correlative imaging methods illustrates the point: microscopy is central to the modern biosciences and is helping to integrate subfields—in this case, structural biology and molecular biology [3]. It is no coincidence that high-impact papers dealing with correlative imaging continue to proliferate, even though this avant-garde vision of combining cellular and molecular tools has been around since the early 1970s. Many advances in the clarification of structure–function relationships have

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occurred when molecular and structural biology techniques were applied concurrently. Classical outcomes from the meeting of morphologists and molecular biologists have included the elucidation of the origin of peroxisomes, the revision of Golgi-transport, the dynamics and structure of proteasomes, the interaction of desmosomal cadherins, and the in-depth revision of the ultrastructure of different cellular components [4].

Complexity — ally or foe?

Microscopes, like the other machinery of science, are becoming increasingly more complicated and one might wonder how this impacts research outcomes. In principle, more sophisticated tools offer scope for greater insights and understanding, yet there is also an increased risk of researchers applying these instruments incorrectly. With this in mind, we like the quote: 'it is important to learn the alphabet first, then to learn to write sentences, and if you are lucky you might one day win the Nobel Prize for literature.' The same philosophy is true for microscopy and microanalysis. Understandably, researchers are attracted to high-end instruments based on the latest vogue in biological research, but they often make the fundamental mistake of not 'learning the alphabet', of not using the most appropriate instrument for their work or of not fully grasping the inherent limitations of a new microscopy technique before applying it. This is due, at least in part, to the buffer provided by advanced software interfaces; though modern instruments are becoming more complicated, the commensurate integration of computers and instruments can sometimes make microscopes seem deceptively easy to use. Without appropriate theoretical insights and practical guidance, researchers, especially molecular biologists, can end up using high-end research platforms less than optimally. These issues can be further compounded by the power of modern visualization, reconstruction and image analysis software, which can cause essential elements in the data to be overlooked or, conversely, allow the data to be over interpreted.

The risk of researchers collecting poor-quality or even artefactual data can be minimized through careful management of high-end microscopes and provision of quality user support and training. In an ideal world, therefore, every Biomolecular Research Institute would be affiliated with the central microscopy facility of their institution, rather than running their own Molecular Imaging Unit. In that way, they would avoid problems in sample preparation, imaging and data processing, and the many possible artefacts that can arise during this multi-step process. Moreover, the expert staff and the different training programs offered by a centralized microscopy facility (see, *vide infra*) can eliminate most misinterpretation of data and/or the publication of artefact-rich images. Mistakes in microscopy are, unfortunately, still frequent and have major budgetary implications on many fronts, such as wasted consumables, instrument downtime, unnecessary staff load and, most importantly, potential ethical issues. Although one can only speculate as to the overall impact and cost of this worldwide, it is clear that the careful collected research equipment and research funds should be nurtured with more care to respect the investments made by tax payers and grant funding bodies.

Centralized microscopy facilities

The ideal approach for supporting researchers in their use of high-end microscopes is through a centralized microscopy facility, in which users' needs are carefully monitored by facility staff throughout the lifetime of the project. For biological projects, it is advisable to start out with overall dynamic observation in time; *i.e.* time-lapse live cell imaging. This allows facility users to first understand what is going on in the live context and then to define the particular point in time where the relevant (sub)cellular phenomena occur. From that moment onwards, the high-end instruments come into play as appropriate, thereby avoiding the incorrect use of advanced machines. Increasingly, this approach will be facilitated by new sample preparation devices that are on the way to bridge the time-resolution gap between light optical microscopes and the high-end electron microscopes by using rapid-sample-transfer preparation systems. Another core element in managing the use of high-end microscopes is the availability of a comprehensive staff profile, blending academics, research associates and technical staff, all with

different training and backgrounds. This unique pool of know-how and skills helps solve the vast majority of issues that arise in the use of advanced microscopy. In addition, quality-training programs—typically offered as short, field-dedicated training courses that blend both theory and hands-on practice—help to ensure that researchers have sufficient expertise to use instruments correctly. These also serve as the first training ground for the next generation of 'biomolecular' structural biologists [5].

As part of the user experience in a centralized microscopy and microanalysis facility, projects should start with a 'new user meeting' in which the research project is discussed in depth with skilled microscopists and technicians. Subsequent attendance at one or more microscopy training courses is usually of value before the (molecular) cell biologist gets hands-on with the microscopes. This approach has proven to be highly successful and productive here in the Australian Key Centre for Microscopy & Microanalysis and guarantees successful outcomes as shown in Ph.D. theses, reports, peer-reviewed journal papers, and so on.

Of course, core microscopy or imaging facilities offer many more advantages than the users experience alone. For example, they have the large user-base necessary to justify substantial government investment in increasingly costly instruments, combined with the staff capital required to take full advantage of such equipment. Centralized facilities also have the capacity to ensure that suites of microscopes work correctly, and that problems are quickly identified and rectified with minimal downtime for the lifetime of the instruments. Furthermore, placing expensive microscopes within such facilities reduces the precious time spent by individual academics and researchers in independently exploring, often in vain, the mechanisms to attract such infrastructure.

Watch the step

Biologists encounter various pitfalls during the collection of microscopy images, though these vary from project to project, user to user, and sample to sample. One should start at the beginning, with the art of sample preparation. The relevant quote here is 'garbage in, garbage out'. Good microscopy starts with proper sample preparation according to the highest international standards, which can be found in relevant literature sources. Sample preparation has been an intriguing and ongoing research topic for decades, but still continues to grow and evolve with the microscopy techniques, illustrating its importance. Often, inexperienced microscopists find it difficult to adapt complex preparatory procedures to the needs of a typical experiment at hand. This is natural given the plethora of factors that can play a key role in the meticulous processing of specimens from a specific tissue or organ: surgical skills, route of perfusion, gravity or pump perfusion, pressure, flow, composition and sequence of perfusion fluids, saline washing, osmotic value, ion composition, quality of chemicals, prelevation techniques, cutting or fracturing methods, the frequency of changing fluids, temperature, and so on. Fortunately, most structural biologists are aware of the importance of good sample preparation and many make it their mission to guide molecular biologists in the fine art of sample preparation.

Another common pitfall occurs at the other end of the process; after acquiring the end product, researchers face the challenge of interpretation and of retrieving the relevant information from those images. It is essential that the molecular biologist and morphologist meet with each other well before this point, and on an ongoing basis, to help avoid subsequent problems in the collection, analysis and interpretation of microscopy data. From our perspective, such meetings are most productive interactions as both parties educate each other about the availability of techniques to dissect cells molecularly and the complementary methods to image and characterize cells at nanometre resolution. This crossover is a key-indicator of high-quality and, ultimately, high-impact research.

The changing fortunes of microscopy

Historically, microscopy and microanalysis were expected to become obsolete once molecular biology tools became readily available for every laboratory. There was even a time when senior academic management looked askance at you if you made the brave

claim of being a microscopist. Of course, these views were just heralds of changing scientific fashions: microscopy was in style in the 1960s and 1970s, but definitely was not popular in the late 1980s and early 1990s [5–6]. Fortunately, this situation has changed in recent years with the introduction of cryo-electron tomography techniques and rapid, live-imaging techniques. A simple scan of websites for academic and scientific jobs shows just how many positions now are available for trained molecular microscopists. Certainly, the Australian experience confirms the essential role of microscopy in modern biological research; Australia's bio-scientists clearly recognize the importance of more advanced molecular image-analysis methods, such as fine-structure immunogold technology and (cryo-) electron tomography [7]. The Federal Government's recent funding of an Australian Microscopy and Microanalysis Research Facility, headquartered here at the University of Sydney, illustrates the high value that policy makers place on this key scientific infrastructure. Ultimately, combining advanced biological microscopy with molecular cell biology techniques is the only way to ensure that research is highly competitive at the international level [8] and, perhaps more importantly, to avoid being limited to molecular biology data alone [6].

It was suggested once at a conference that microscopists have become an endangered species and that it is high time to invest proper funding in preservation programs for them and in the training of the new generation. This is true; quality training is essential to meet the growing demand for molecular microscopists. An important aspect in the integration of structural and molecular biologists is offering them the possibility of doing dedicated training programs in microscopy. At present, however, there are only a few dedicated training programmes on offer on the international scene. There is no doubt that Australia is a global leader in this area. For example, the University of Western Australia runs a unit in electron and optical microscopy for undergraduate students in biology, engineering, and materials and earth sciences. The University of Sydney offers full postgraduate courses in microscopy and microanalysis, as well as doctoral research programs. This training capacity goes some way to meeting the local demand for skilled molecular microscopists in Australasia, and some of the fresh graduates end up taking jobs in the United States and Europe.

Merging cellular and molecular biology at the microscope column

Modern advanced biomolecular microscopy comprises the full range of instruments that explore specimens with light, lasers, soft x-rays, electrons or scanned probes to generate images and spectra at resolutions from the microscale towards the atomic scale. Based on the increasing availability of different imaging techniques and the complexity of the field, there is a tendency for biologists to hand over all the imaging, including sample preparation, to dedicated microscopists. Unless this is done in a fully collaborative manner, handing over the microscopy 'chores' will never give the best research outcomes. Though there is an undisputed increase in the complexity of microscopes, this is true for every research field (such as proteomics, gene chip analysis etc.) as newer, faster, and more complicated instruments find their way into research laboratories. Some might see this as further evidence for the need for more dedicated microscopists to take over the imaging tasks from the molecular biologists. However, this could create the worst-case scenario in which 'trained specialists' operate the microscopes without any underlying knowledge or understanding of the biology of the samples they are looking at. Researchers can only get the maximum out of their microscopy data with a full understanding of (*i*) the sample preparation, (*ii*) the operation of the microscope and (*iii*) how the data were collected. At the end of the day, the people best placed to interpret the final images are the researchers themselves. The optimal approach to research with advanced molecular microscopy is a collaborative one. When combined with regular training and up-skilling, such a collaborative approach generates the highest-quality research and forms an ideal setting for training biologists in new technologies. A core task for the next-generation microscopist is to navigate the inexperienced researcher through the ocean of techniques, fully informed of the strengths and limitations of each approach, thereby avoiding generation of 'false results' in the complex world of microscopy and microanalysis!

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