Light Microscopy Imaging Facilities

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Historically core imaging facilities have been associated with electron microscopes. With the very widespread use of fluorescent markers for both fixed and living cells and tissues the light microscope facility has emerged in its own right. Well-run core light microscope imaging facilities are focal points within research institutions. Researchers want to do science, acquire images and manipulate data for publication; for them, the microscope and the computing infrastructure within the imaging facility are merely tools. Staff provide organization, continuity, tuition and knowledge and maintenance of equipment, which encompass the needs of both novice and advanced users. Increasingly, imaging facilities are considered from the outset as a component in the design of a new research building, and must recover the costs of operation. This article discusses the need for optical sectioning, the role of the light microscopy facility and points to consider in its design.

Introduction

We are a visual species, and our ability to observe and record images using microscopes has undergone a quantum leap. In the past twenty five years, light microscopy has benefited tremendously from advances in computing, fluorescent stains and laser technology. These disparate fields have been combined in a fortuitous union to bring about a renaissance, giving rise to the complex instruments now routinely employed in a modern light microscopy facility.

Fluorescence microscopy (Lichtman and Conchello, 2005; Waters and Swedlow, 2007; Waters, 2009) is one of the most widely used tools in modern biomedical research and has revolutionized the way in which we view the microscopical world. Fluorescent probes are available in a wide range of colours that span the visible spectrum and beyond into the near infrared, and genetically encoded fluorescent proteins (Shaner *et al.*, 2007; Shaner *et al.*, 2005; Davidson and Campbell, 2009) have enabled us to observe specific proteins in living cells in real time. There are several advantages in using fluorescent probes, but they are self-luminous, and hence cells and tissues must be optically sectioned (Conchello and Lichtman, 2005; Murray, 2005) or deconvolved (McNally *et al.*, 1999; Parton and Davis, 2004) to give images with good signal-to-noise ratios (SNR), and free of blur. The microscopes capable of these optical sectioning and live-cell imaging techniques are specialized and expensive. Historically, electron microscopes have been installed into central core facilities largely because of their sophisticated design, expense and labour-intensive operation. Such is the complexity and expense of the modern light microscope now employed for biological research, that university departments and institutions increasingly pool their resources to fund and operate these instruments within centralized facilities for access to equipment that otherwise would be beyond the scope of individual research groups to support. **See also**: Deconvolution Fluorescence Light Microscopy; Electron Microscopy; Fluorescence Microscopy; Green Fluorescent Protein (GFP)

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Image facilities vary enormously, according to the equipment, funding, policies and research needs of the host university or institute. Some imaging facilities incorporate both light and electron microscopes together with flow cytometers; others house light and electron microscopes separately. Light and electron microscopy both entail (a) image acquisition, (b) data handling and (c) image manipulation and analysis and because of their complexity require specialist operation and support. Most specialized imaging facilities evolve in an ad hoc fashion as the need for their existence arises, once the funding is in place, and as equipment is bought. The character and function of a core microscope imaging facility is very largely defined by the user base that they serve, and the specialist staff who operate them.

Anything more than the simplest imaging facility will necessitate specialist rooms being adapted or built from scratch. Once built, it is difficult to reverse this process, to accommodate unforeseen consequences, and with design the devil is in the detail. Books, papers and articles of long-standing have considered the design and function of electron microscopy core facilities (see Anderson *et al.*, 2007), but there has been relatively little published about the role of light microscopy imaging units, and the points to consider when designing and installing a dedicated core facility.

Advantage of Fluorescence Contrast Enhancement

Untreated or living tissue generally lacks contrast to a significant degree, and biological specimens normally require contrast enhancement so that the fine detail inherent in their structure can be distinguished and resolved against the background, to be studied and understood. Most brightfield microscopical contrast techniques only permit up to approximately 40% enhancement for visibility and resolution of detail. The value of fluorescence microscopy lies in the fact that, unlike these other modes of optical microscopy that are based on macroscopic specimen features (such as phase gradients, light absorption and birefringence), fluorescence microscopy is capable of imaging with very high contrast and visibility (**Figure 1**). The distribution of a single molecular species can be accurately determined based solely on the properties of fluorescence emission. Furthermore, the specificity and sensitivity of antibody-conjugated probes and genetically engineered fluorescent protein constructs allows multiple labelling and the precise location of intracellular components labelled with specific fluorophores, which can be monitored over time, as well understanding as their associated diffusion coefficients and interactions with other biomolecules. **See also**: Differential Interference Contrast Light Microscopy; Immunofluorescence; Light Microscopy – Brightfield and Darkfield Illumination; Phase Contrast Microscopy

Optical Sectioning Approaches

Depth of field is the axial depth of the space on both sides of the object plane within which the object can be moved without detectable loss of sharpness in the image. Depth of focus is the axial depth of the space on both sides of the image plane within which the image appears acceptably sharp while the positions of the object plane and of the objective are maintained. A high-magnification objective (because of its large numerical aperture, NA) has an extremely limited depth of field, yet relatively large depth of focus. The converse is also true for low NA (low-magnification) objectives.

Because fluorophores are self-luminous, and are generally distributed throughout the sample, illuminating the sample will cause it to fluoresce throughout its entire thickness regardless of where the objective is focused along the optical axis. Since the depth of field of the objective may be 800 nm or less, and a typical cell from 5 to 15 μm thick, then over 80% of the signal from the specimen may well be out-of-focus blur (**Figure 2**). Additionally, the emitted fluorescent signal may be scattered (refracted, reflected and diffracted) by components of the specimen on its return to the objective, and appear to come from nearer the surface of the specimen than is actually the case. The result is obscuring of the true in-focus signal by out-of-focus blur, and a significant decrease in the SNR.

There have been several approaches to optical sectioning to 'clean up' the fluorescent signal and deal with the out-of-focus fluorescent blur in the image. These are listed in **Figure 3**. These are (a) point-scanning and spinning-disc laser-scanning confocal microscopy, (b) structured illumination microscopy, (c) widefield deconvolution microscopy, (d) multiphoton microscopy and (e) total internal reflection microscopy (TIRF). Each approach complements the others in the type of information and data that can be extracted from the specimen. Most light microscope facilities, however small, will at least have a point-scanning laser-scanning confocal microscope. **See also**: Fluorescence Microscopy

Choice of Microscope

The particular microscope that is suitable for a specific imaging task depends on a number of factors (**Table 1**), such as

- How the cells will be presented to the microscope (slides, dishes and multiwell plates)?
- Whether samples are fixed or live, watery, thick or thin.
- Fluorescence-conjugated labels, fluorescent proteins and/or brightfield imaging.
- How long do you wish to image for (cell maintenance techniques; bleaching; photo-toxicity)?
- Whether photo-bleaching for kinetic experiments is required (FRAP and FRET).
- Whether spectral unmixing of closely apposed fluorescent proteins needed to avoid bleed through.

Figure 1 Advantages and disadvantages of fluorescence. PMT, photo-multiplier tube.

In general, for thick specimens (greater than 10 μm) use a confocal microscope; for thin ones, capture all the signals with a widefield microscope rather than attenuating with a confocal pinhole, and deconvolve. For speed, and for recording dynamic events in living cells, use a Nipkow spinning disc system (Nakano, 2002; Gräf *et al*., 2005) and for deep imaging (greater than 100 μm) use a multiphoton microscope (Helmchen and Denk, 2005; Stutzmann and Parker, 2005; Niesner *et al*., 2008). For studying very fast dynamics at the cell membrane, right up at the surface of the coverslip, use TIRF (Axelrod, 2001; Groves *et al*., 2008). Laser-scanning confocal microscopes provide better control of bleedthrough and those fitted with spectral detectors can be used to discriminate between fluorescent proteins whose emission spectra closely overlap. Widefield microscopes use charge-coupled device (CCD) cameras as detectors, and so give a higher SNR for weak fluorescent signals from thin samples (Swedlow *et al*., 2002; Andrews *et al*., 2002). An example of the poster available within the Light Microscopy Facility at the University of Sheffield to help users decide which microscope to use for their work is shown in **Figure 4**.

Design of the Imaging Facility

There are five major design points to take into account, of which vibration isolation and temperature stability (for time-lapse experiments of any duration) are crucial.

- Vibration isolation
- Temperature and humidity stability
- Local lighting
- Data networking
- Function work-flow within the facility

Vibration isolation can be by compressed air piped into the room, or miniature compressors to operate the anti-vibration tables. Computers, heating ducts and monitor

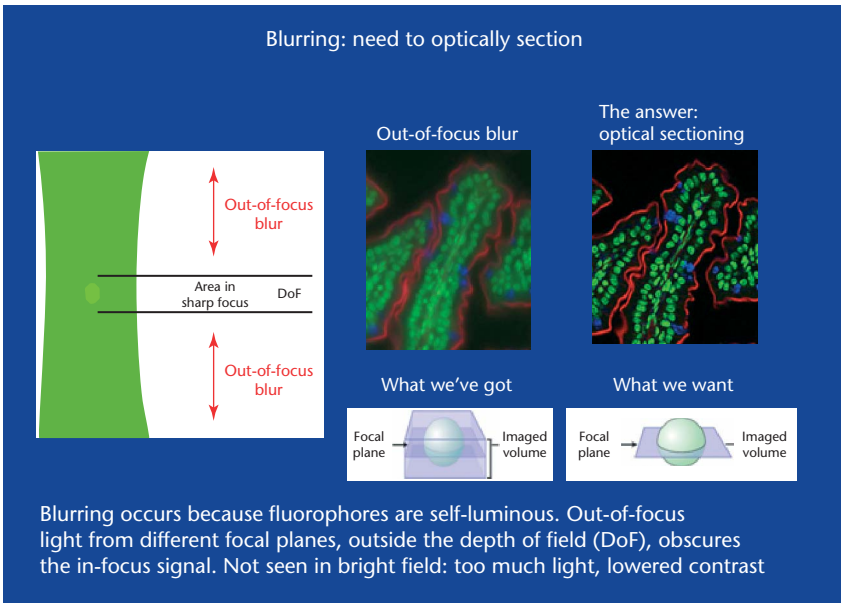

Figure 2 Schematic representation for why we need optical sectioning.

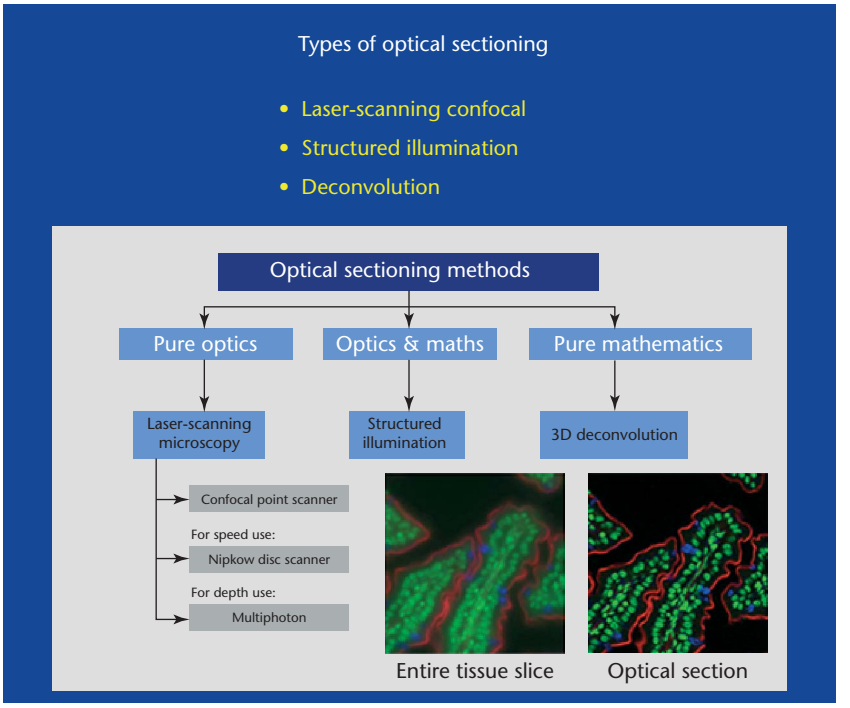

Figure 3 Schematic representation of the different types of optical sectioning.

worktables should be isolated from the microscope. Air conditioning units should not be positioned above microscope workstations, but it should be installed with sufficient flexibility to give good ventilation and temperature control with a tolerance of $\pm$ 1°C to give meaningful stage stability (Adler and Pagakis, 2003; Lee *et al.*, 1996). It is best to have small rooms that can be individually tightly controlled for temperature, rather than curtained-off cubicles as part of a larger room space. Calculate the expected heat exhaust of the microscopes, lamps, lasers and live-cell incubators and allow 0.5–1 kW for each user. Local light should preferably have both local low-level red lighting as well as rheostat controlled white light (either angle-poise or wall-mounted) for individual cubicles or rooms. For data networking and

Table 1 Comparison of different types of microscope within the BMS-MBB Light Microscopy Facility

Type	Example	Advantages	Disadvantages	Typical application
Point-scanning confocal	Leica SP5 upright	Good optical sections	Bleaches	Thick well-stained cells and tissues 2–200 μm
	Olympus FV-1000 upright	Laser switching	Noisy	Bleedthrough correction
	Zeiss LSM 510 inverted			Bleaching kinetic studies
				Co-localization studies
Widefield deconvolution	DeltaVision DV-1 (LMF)	Collects all weak signals	Slow	Thin cells and tissues 1–20 μm
	DeltaVision DV-2 (MBB)	No laser illumination	Deconvolution is time-consuming	Sensitive live cell imaging
		Measured PSF-accurate		Co-localization studies
	Olympus BX-61 Optigrid	Calculated PSF-quick		
Structured illumination	Olympus BX-61 Optigrid	No laser illumination	Can leave stripes	Quick confocal sections
		Easy and quick to use		CCD for sensitive samples
Spinning-disc confocal	Perkin Elmer UltraVIEW VoX	Fast: high frame rate weaker lasers; EM-CCD	Fixed pinhole size	For high frame rate >5 fps
			Noisy	Bleaching kinetic studies
Widefield live cell	Leica DM-IRBE live-cell	Long W-D objectives	Limited fluorescence filters	For long-term time-lapse
		Xenon illumination		Sensitive live cell imaging FP-labelled living cells

Notes: EM-CCD, electron multiplying charge-coupled device; PSF, point spread function and W-D, working distance.

electrical requirements, you always need more sockets and network ports than you think.

Where possible the functional workflow of people into the facility should keep away from microscope workstations (to avoid draughts). It should be made easy to transport specimens from users' laboratories to the core facility, or have access close to specimen preparation and cell culture facilities. As a minimum, a wet-bench and sink should be provided within the light microscope facility with a small incubator and piped carbon dioxide to support live-cell imaging. Multiphoton microscopes will need other gas services, such as filtered helium supply for drying/purging Ti-S IR lasers. Finally, you will need sufficient storage space, and this can be provided by shelving behind each workstation to set transformers, lamps, shutter units carbon dioxide controllers and suchlike, as well as safety manuals and folders and logbooks.

Safety Issues

Most microscopes which incorporate laser illumination are sold as safe by engineering design, with sealed fibres, so that the laser emerges divergent from the objective. However, some TIRF microscopes may be hazardous, so it is imperative to identify these hazards, and assess the risk to those who may be working with the microscopes, whether users, staff or service engineers. Have in place a series of standard operating procedures, sufficient user-training and safety documentation which is signed off by users. Other potential hazards are presented by electrical equipment, particularly where (e.g. laser units) such equipment may be water cooled. There should also be sufficient storage for flammable cleaning solvents (e.g. petroleum ether and ethanol), and control of substances hazardous to health substances (COSHH) (e.g. stains and fluorophores).

Requirements within a light microscopy imaging facility

The distinction can be made (Anderson *et al*., 2007) between a light microscopy *service* where staff generate data for a more passive user base, and a *facility* where staff help users actively acquire their own images and generate data. Generally, tighter control of a service means that it is possible to maintain core equipment in better condition, although for a large number of users and microscope systems, the service approach, which is staff intensive, may become untenable. Depending on the size of the facility, there may be one or more staff. It is not unheard of to have a

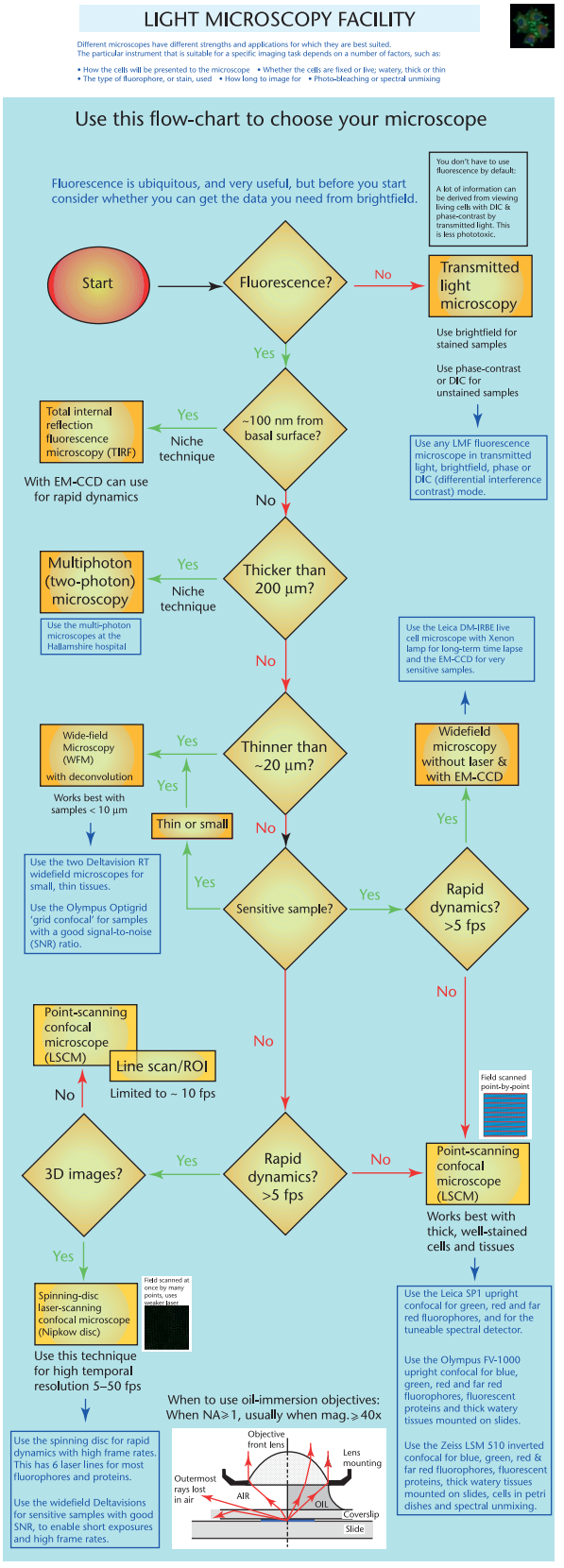

Figure 4 Poster to show facility users which microscope to use.

single member of staff for seven or eight microscopes, although in a busy facility, a ratio of one staff member to three or four microscopes is more reasonable. This may seem like overkill, but when a machine goes wrong, and must be brought back into service with the minimum of down-time then this is not, in fact, the case. Some microscopes may receive only occasional use, but they require full-time attention if software or hardware malfunctions just when they are needed.

Role of the Support Staff

The chief requirement of the staff of an imaging facility is to be approachable, so that users are confident to use the light microscope facility (LMF), ask questions and operate the microscopes effectively. Users encompass a broad range of personalities, but most are aware that they need to be trained to use sophisticated and complex equipment that they may not have encountered before (Nature Editorial, 2007). Staff in an imaging facility ideally require five key attributes: approachability, knowledge, flexibility, capability and experience. People have to be comfortable in walking through the door of the imaging facility to speak to 'expert' staff, who in turn need the knowledge to answer those questions, the ability to give pertinent advice and the flexibility to relate to a broad spectrum of users from the over-bearing to the under-confident. They also need to be capable of problem-solving whether it may be in experimental design or keeping complex mechanical and optical equipment functioning. It is also helpful to know colleagues and trade representatives, so that present needs and future trends can be anticipated and catered for.

Another point to consider is whether the imaging facility will provide a service for mounting media, antibody stains and reagents. Consistency in specimen preparation means that users are less likely to waste their time trying to collect scientifically rigorous data from mediocre or poor specimens. All this needs is a fridge-freezer somewhere, and bench space to aliquot out stocks.

Microscopy Teaching Role

Teaching the principles of microscopy and educating users is an important part of the job of the light microscopy facility staff. No longer are biologists merely taking snapshots of illuminated slices of dead tissue, but are increasingly investigating living tissue in three or more dimensions. This is where the imaging facility staff have a key role to play as specialist advisers and educators. Do the imaging facility staff carry out most or all of the image acquisition and analysis, or are these skills taught to the users themselves? Are the support staff involved in teaching the principles of microscopy in a wider sense, or is their role restricted to running the imaging facility? Broadly speaking, unless the project is very short and the microscopy unit is very small, it is more effective to train the researcher to

carry out their image acquisition – they should know better than anyone what questions they want answering, and how the final images and data analysis should support their hypotheses. Equally, there is a benefit in teaching the principles of image formation, so that researchers can *understand* what they are doing when operating the microscope. At the very least users need not only to know how to use a particular microscope, but also about how microscopes form images, how fluorochromes work, how cameras and detectors acquire images and the fundamentals of image processing and data handling. For this reason, most users recognize the value of microscopy tuition, and it is not uncommon for image facility staff to have over-subscribed teaching courses. Generally, specialized time-consuming techniques, such as FRET (Förster resonance energy transfer) are not conducive to core facilities, but lend themselves best to individual research groups who can invest the time and learning required to become proficient in the technique.

IT Support and Data Handling

Good data storage and IT support is essential for an imaging facility to function effectively, and this is particularly so now that light microscope images and data contribute to multifactorial data analysis. It is better to keep image acquisition and processing within a single facility, since these functions are intimately related (Chi, 2008; Megason and Fraser, 2007; Kherlopian *et al*., 2008). Good high-speed networking and large storage capacity on a dedicated redundant array of independent disks (RAID)-array server is pretty much mandatory. Try to install LMF computers behind a dedicated firewall. With the increasing advent of large 3-, 4-, 5- and 6D datasets (x,y,z, lambda (λ), time, multiple points) and the holistic interpretation of images along with molecular biology and proteomic data (high-throughput), efficient data naming, transfer and manipulation for analysis is essential to efficient research. As a minimum, consider instigating a facility- or departmental-wide standard nomenclature for data folders of images (e.g. year_date_name_microscope: 2009_09_23_HarryW_LSM510). This makes them much easier to locate, retrieve and work on. There has been development of multifactorial data-handing databases (Peng, 2008; Goldberg *et al*., 2005; Gustafson *et al*., 2007) for handling data from light microscopes and high-throughput systems together, and preserving the metadata associated with the original experiment.

Consider also the number of the instruments and the software complexity in the facility. Having different instruments from different manufacturers means that, in a large multiuser facility, people can capitalize of the design strengths (protected by patents) of one microscope or another. They may also prefer the software operating package of, say, one point-scanning confocal compared to another in the facility. Conversely, there is an argument for streamlining on microscopes from one manufacturer, for most will have one image acquisition software package that can be used to operate several, or all, microscopes in the unit. This helps to ensure that a mature user base develops that much more quickly. Experienced users are essential to the smooth operation of the imaging facility. There are thus fewer interruptions and sudden issues to be dealt with – 'fire-fighting' as we call it. Teaching, support maintenance and charging – the daily life of the image facility staff – can then be carried out much more effectively. Also have a dedicated LMF list server – a mailing list for internal communication with users.

Booking Database

An electronic database can be useful in all but the smallest units for people to equitably book time on the microscope. Even some large facilities will keep a paper-based diary in lieu of an electronic scheduler, which facility staff keep and monitor. This has the advantage that users have to turn up in person to explain and define what they want to do. This in turn gives a greater chance to monitor image acquisition, ask questions, be interested and generally help users in getting the images and data that they require. The advantage of an electronic system is that mature users of the facility, who have a very clear idea of what images they want to acquire and how to go about doing so, can book their sessions on the microscope remotely from their own workstations without having to travel and find the imaging facility staff. Which approach is best will depend on the size of the imaging facility and the staff and users. We use the LMF scheduler from Scionics Computer Innovation GmbH (http://www.scionics.de); another option is to use the Pasteur/Rockefeller Platform Management System (PPMS; http://www.ppms.info). Alternatively, write a custom programme in-house, or use a free software option (e.g. http://sourceforge.net/projects/mrbs/).

Instrument Maintenance and Cost Recovery

Staff salaries and instrument maintenance contracts are both essential and very expensive. Well-trained staff can pre-empt major equipment failures, or carry out small repairs to keep microscopes in serviceable order. It is false economy to try and run an imaging facility without any form of service maintenance cover for when things inevitably go wrong and equipment fails. Only infrequently used microscopes which are no more than a routine fluorescence microscope should be considered for leaving off a service contract, and in that case the cost of a call-out to the engineer when things go wrong should be factored into the operating costs. Lasers are expensive and replacing and aligning them very time-consuming. A service contract will cost several thousands of pounds annually, but it generally pays for itself within the course of the year.

Other costs that may need to be factored into running an imaging facility include instrument depreciation over a specific time (usually 10 years), the cost of room space or hire, electricity, supply of carbon dioxide for live-cell incubation chambers, and the cost of replacement lamp and fluorescence illuminators, disposable consumables and reagents. A good toolbox is indispensable, and the cost of other small equipment items such as pipettes, fridges and cell culture incubators need to be paid for. Whether these costs are found from initial start-up grants, or must otherwise all be factored into a charge levied to users for operating the microscopes is a policy decision. Very few imaging facilities are in the fortunate position of not having to charge for their services, and it helps to have a clear and simple accounting system to manage the costs that must be invoiced to the users.

Where to Go for Help

Other professional microscope imaging facility support staff and trade representatives are a valuable resource. There is no substitute for experience, but speaking to your opposite number at another institution or visiting them, if local, can often prevent you wasting time 're-inventing the wheel'. An extension of this network of colleagues is provided by two listservs: the confocal listserv (http://www.microscopy-online.com/confocal.shtml) and the microscopy listserv (http://www.microscopy.com). There are also specialist societies, such as the Royal Microscopical Society (http://www.rms.org.uk), the European Light Microscopy Initiative (ELMI) (http://www.embl.org/elmi) and the Microscopical Society of America (http://www.microscopy.org) to name but three, as well as national initiatives (e.g. http://www.bioimaginguk.org/index.php/Main_Page) in the UK to link facility managers and staff together.

Do not under-estimate the body of knowledge and experience that trade representatives can offer you. It is in their interest to help support the equipment that you bought from their company – welcome them and use them. They are specialist staff, not door-to-door salesmen. A further resource is the microscopy primer website (http://micro.magnet.fsu.edu/primer) that covers most topics in depth. Although the internet is not peer-reviewed, this website is reliable, useful and is supported by some of the major light microscope manufacturers. It is also worth looking at manufacturers' websites for the support literature and webpages that they host in their own right.

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References

Adler J and Pagakis SN (2003) Reducing image distortions due to temperature-related microscope stage drift. *Journal of Microscopy* **210**(2): 131–137. doi: 10.1046/j.1365-2818.2003.01160.x.

Anderson KI, Sanderson J and Peychl J (2007) Design and function of a light microscopy facility. In: Shorte SL and Frischknecht F (eds) *Imaging Cellular and Molecular Biological Functions*. Berlin: Springer, ISBN-13: 978-3-540-71330-2.

Andrews PD, Harper IS and Swedlow JR (2002) To 5D and beyond: quantitative fluorescence microscopy in the post-genomic era. *Traffic* **3**(1): 29–36. doi: 10.1034/j.1600-0854.2002.30105.x.

Axelrod D (2001) Total internal reflection fluorescence microscopy in cell biology. *Traffic* **2**: 764–774. doi: 10.1034/j.1600-0854.2001.21104.x.

Chi KR (2008) Imaging and detection: focusing on software. *Nature Methods* **5**(7): 651–658. doi: 10.1038/nmeth0708-651.

Conchello J-A and Lichtman JW (2005) Optical sectioning microscopy. *Nature Methods* **2**(12): 920–931. doi: 10.1038/nmeth815.

Davidson MW and Campbell RE (2009) Engineered fluorescent proteins: innovations and applications. *Nature Methods* **6**(10): 713–717. doi: 10.1038/nmeth1009-713.

Goldberg IG, Allan C, Burel JM *et al*. (2005) The Open Microscopy Environment (OME) Data Model and XML file: open tools for informatics and quantitative analysis in biological imaging. *Genome Biology* **6**: R47. doi: 10.1186/gb-2005-6-5-r47 http://genomebiology.com/2005/6/5/R47.

Gräf R, Rietdorf J and Zimmermann T (2005) Live cell spinning disk microscopy. In: Rietdorf J (ed.) *Advances in Biochemistry and Engineering Biotechnology*, vol. 95, chap. 7, pp. 57–75. Berlin: Springer, ISBN = 3-540-23698-8.

Groves JT, Parthasarathy R and Forstner MB (2008) Fluorescence imaging of membrane dynamics. *Annual Review of Biomedical Engineering* **10**: 311–338. doi: 10.1146/annurev.bioeng.10.061807.160431.

Gustafson C, Bug WJ and Nissanov J (2007) NeuroTerrain – a client-server system for browsing 3D biomedical image data sets. *BMC Bioinformatics* **8**: 40. doi: 10.1186/1471-2105-8-40.

Helmchen F and Denk W (2005) Deep tissue two-photon microscopy. *Nature Methods* **2/12**: 932–940. doi: 10.1038/nmeth818 Corrigendum: *Nature Methods* **3**(3): 235. doi: 10.1038/nmeth0306-235.

Kherlopian AR, Song T, Duan Q *et al*. (2008) A review of imaging techniques for systems biology. *BMC Systems Biology* **2**: 74. doi: 10.1186/1752-0509-2-74.

Lee MF, Kong SK, Fung KP, Lui CP and Lee CY (1996) Practical considerations in acquiring biological signals from confocal microscope: solvent effect and temperature effect. *Biological Signals* **5**(5): 291–300. doi: 10.1159/000109202.

Lichtman JW and Conchello J-A (2005) Fluorescence microscopy. *Nature Methods* **2**(12): 910–919. doi: 10.1038/nmeth817.

McNally JG, Karpova T, Cooper J and Conchello J-A (1999) Three-dimensional imaging by deconvolution microscopy. *Methods* **19**: 373–385. doi: 10.1006/meth.1999.0873.

Megason SG and Fraser SE (2007) Imaging in systems biology. *Cell* **130**(5): 784–795. doi: 10.1016/j.cell.2007.08.031.

Murray JM (2005) Confocal microscopy, deconvolution, and structured illumination methods. In: Goldman RD and Spector DL (eds) *Live Cell Imaging: A Laboratory Manual*, chap. 14, pp. 239–279. New York: CSHL Press, ISBN-10: 0-87969-683-4.

Nakano A (2002) Spinning-disk confocal microscopy – a cutting-edge tool for imaging of membrane Traffic. *Cell Structure and Function* **27**(5): 349–355. doi: 10.1247/csf.27.349.

Nature Editorial (2007) Under the microscope. *Nature* **447**(7141): 116. doi: 10.1038/447116a.

Niesner RA, Andresen V and Gunzer M (2008) Intravital two-photon microscopy: focus on speed and time resolved modalities. *Immunological Reviews* **221**: 7–25. doi: 10.1111/j.1600-065X.2008.00582.x.

Parton RM and Davis I (2004) Lifting the fog: Image restoration by deconvolution. In: Celis JL (ed.) *Cell Biology: A Laboratory Handbook*, 3rd edn, chap. 19. San Diego: Academic Press, ISBN-13: 978-0-12-164733-1.

Peng H (2008) Bioimage informatics: a new area of engineering biology. *Bioinformatics* **24**(17): 1827–1836. doi: 10.1093/bioinformatics/btn346.

Shaner NC, Steinbach PA and Tsien RY (2005) A guide to choosing fluorescent proteins. *Nature Methods* **2**(12): 905–909. doi: 10.1038/nmeth819.

Shaner NC, Patterson GH and Davidson MW (2007) Advances in fluorescent protein technology. *Journal of Cell Science* **120**(24): 4247–4260. doi: 10.1242/10.1242/jcs005801.

Stutzmann G and Parker I (2005) Dynamic multiphoton imaging: a live view from cells to systems. *Physiology* **20**: 15–21. doi: 10.1152/physiol.00028.2004 1548-9213/05.

Swedlow JR, Hu K, Andrews PD, Roos DS and Murray JM (2002) Measuring tubulin content in *Toxoplasma gondii*: a comparison of laser-scanning confocal and wide-field fluorescence microscopy. *Proceedings of the National Academy of Sciences of the USA* **99/4**: 2014–2019. doi: 10.1073/pnas.022554999.

Waters JC and Swedlow JR (2007) Interpreting fluorescence microscopy images and measurements. In: Zuk D (ed.) *Evaluating Techniques in Biomedical Research*. Cambridge, MA: Cell Press, http://www.cellpress.com/misc/ETBR.

Waters JC (2009) Accuracy and precision in quantitative fluorescence microscopy. *Journal of Cell Biology* **185**(7): 1135–1148. doi: 10.1083/jcb.200903097.

Further Reading

Fernández-Suárez M and Ting AY (2008) Fluorescent probes for super-resolution imaging in living cells. *Nature Reviews. Molecular Cell Biology* **9**(12): 929–943. doi: 10.1038/nrm2531.

Frigault MM, Lacoste J, Swift JL and Brown CM (2009) Live-cell microscopy – tips and tools. *Journal of Cell Science* **122**: 753–767. doi: 10.1242/jcs.033837.

Hibbs AR (2004) *Confocal Microscopy for Biologists*. Berlin: Plenum Publishers. ISBN-13: 978-0306484681.

Jepson MA (2006) Confocal or Wide-field? A guide to selecting appropriate methods for imaging. In: Stephens D (ed.) *Cell Imaging*, chap. 2, pp. 17–48. Oxon: Scion Publishing, ISBN-13: 978-1904842267.

Khodjakov A and Reider CL (2006) Imaging the division process in living tissue culture cells. *Methods* **38**(1): 2–16. doi: 10.1016/j.ymeth.2005.07.007.

Klaunberg BA and Davis JA (2008) Considerations for laboratory animal imaging center design and setup. *Institute of Laboratory Animal Research Journal* **49**(1): 4–16.

Miyawaki A (2008) Green fluorescent protein glows gold. *Cell* **135**(6): 987–990. doi: 10.1016/j.cell.2008.11.025.

Murphy JA (2002) Designing an electron microscopy facility: step by step procedure. *Microscopy Today* **10**: 36–39. With checklist URL: http://www.sjdccd.cc.ca.us/dept/electmicro/images/Murphy_Design_CheckLsts.pdf.

North AJ (2006) Seeing is believing? A beginners' guide to practical pitfalls in image acquisition. *Journal of Cell Biology* **172**(1): 9–18. doi: 10.1083/jcb.200507103.

Pearson H (2007) The good, the bad and the ugly. *Nature* **447**(7141): 138–140. doi: 10.1038/447138a.

Petty HR (2007) Fluorescence microscopy: established and emerging methods, experimental strategies, and applications in immunology. *Microscopy Research & Technique* **70**(8): 687–709.

Spector DL and Goldman RD (eds) (2005) *Basic Methods in Microscopy*. Oxon: Scion Publishing, ISBN-13: 978-0879697518.

Stephens D (ed.) (2006) *Cell Imaging*. Bloxham: Scion Publishing Ltd, ISBN 1-904842-046.