TECHNICAL

TECHNICAL NOTE 12

Setting up and managing a biological laser scanning microscope resource

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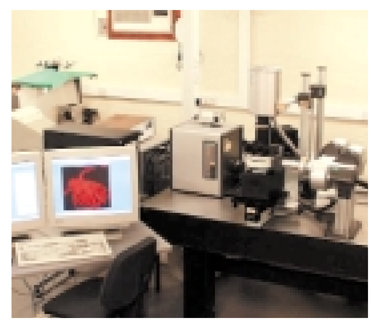
Introduction



Many laboratories recognise that sophisticated microscopes, like other equipment, can deliver maximum value-for-money as part of a central resource which can combine specialist and core services. It is often advantageous to have instruments close to specialist labs (e.g. for containment or special procedures), but organised into a logical resource that delivers the benefits of central support. Instruments (and their users) can be effectively managed, even in remote locations, and supported by a distributed team. This team is tasked with delivering the technology to users in the most appropriate and accessible form. This Technical Note shares our experience in setting up a central resource for Laser Scanning Microscopy.

Getting started

Sell the idea to prospective users by getting a ‘core’ of projects to ‘buy in’ to the scheme. Decide if the equipment will be ‘multi-application’ as well as ‘multi-user’ as the former must be freed, and may need reconfiguring, after each session. Single-application systems, even with many users, can be left set-up with other components over many sessions. Technical support may be supplied by the project team(s).

Funding bodies favour equipment that enhances local and national infrastructure, but you must ensure sufficient time is made available for core projects. Carefully scrutinise each for required resources.

Time should be allocated for:

- Projects (typically up to 1000 hours per year)
- Maintenance (e.g. 4 hrs per week to clean, check performance and maintain data storage.)
- Development (if appropriate – but this must be managed to minimise impact on users)
- Service (a service contract and annual manufacturers checks are essential)
- Demonstrations
- Training (users are best trained incrementally while working with their own samples).

A seminar or workshop is a good way to launch the scheme and to get an idea of the work that must be supported. Full details should be obtained of all relevant research proposals and requests for funds.

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Figure 1 Overall views of the microscope area in the BMU.

Space and refurbishment

Space is needed to operate systems conveniently and safely, often in subdued light. Try to house low-light instruments in a separate room or, alternatively, use low voltage 'pot' lamps (with illumination angles ≥ 10 deg). Use dimming controls for lighting, ideally near each workstation, and shield microscope specimen stages without covering the whole system.

Figure 2 Lighting and power at the BMU.
Main fluorescent room lighting (left) is replaced by track-mounted spot lights controlled by independent circuits and dimmers. Services and lighting controls are mounted on posts (right) adjacent to each workstation. This post shows the dimmer control (top), three standard 13 pin (UK) power sockets and three non standard sockets (lower), which use plugs put only on lasers or PCs, on separate circuit breakers. Other services (e.g. network connections, earth terminals, compressed air, etc) can also be mounted here.

Each microscope should be installed on a sturdy table with free access all around (≥ 0.5m) (*see Fig 1*). Trailing cables are minimised by post-mounted services (*Fig 2*), which should include:

- Electric power (arranged in several circuits with independent earth leakage trips).
- ‘Clean’ electrical power for all lasers, using non-standard plugs to bar other devices.
- A ‘clean’ earth connection for grounding AV tables, Faraday cages etc.
- Compressed air (dry and 5 µm-filtered from line or oil-free compressor (*Fig 3*).
- Computer network connection(s).

Anti vibration (AV) tables are usually required, especially if the resource is not in a basement.

Figure 3 Compressed air.
Optical tables can be floated by a service air supply or from a cylinder as used at UWCM. About 80 PSI is needed. At the BMU, no air supply is available and a small compressor is used instead of a cylinder. This also provides filtered and dried air for purging the TiS laser.

Figure 4 Heat management and air conditioning.
A pair of basic thermostatically controlled air conditioners (one is shown top left) together with a filtered make-up air unit (lower left) bring in fresh air and a ducting system (lower right) with additional extractor fan removes heat from laser cooling fans. The UWCM system (top right) uses a sophisticated +/– 1°C unit with remote control, achieving an extremely stable environment.

Air conditioning capacity (to remove heat and stabilise the room temperature) is determined by the total electrical power for all devices plus 0.5 kW from your users!). Thermostatically controlled units are best (*Fig 4*). Humid air from outside a building increases the humidity and the need for dry-purging of infra-red lasers operating above 870 nm. Dust filters should be fitted to air-conditioning units and cleaned regularly. Try to position microscopes away from air conditioning vents. Laser cooling fans should be ducted out of the room with an additional fan if the length of duct is extended (*Fig 4*).

System specific requirements (electricity, air conditioning, etc.) are included in preinstallation manuals provided by Bio-Rad.

Lab support area

An area separated from the microscopes should be set aside for basic lab space (*Fig 5*). Keep minimum stocks of reagents and consumables, encouraging users to prepare samples with their own materials. A small cart is useful to move materials, perfusion apparatus etc., between lab and microscopes.

This area should (ideally) include:

- Separate sinks for washing glassware, etc. and hands.
- Enough bench space for anticipated numbers of simultaneous users.
- Small incubator for short-term storage of cultures, tissue etc.
- Basic equipment for slide preparation.
- A standard transmission and fluorescence microscope for assessing specimens.
- A dissecting microscope for preparing tissue explants etc.

Figure 5 Support lab facilities.
These can be basic, such as the BMU mini-lab (upper left) or side bench in the UWCM LSM lab (lower left) or more sophisticated (upper right shows the UWCM microscopy tissue culture facilities) depending on the work supported by the unit. Plastic carts (lower centre) are useful for moving equipment between lab and LSM systems and a basic microscope (e.g. lower right) is useful for checking specimens during preparation.

Health and safety considerations

The final plan should be approved by the local safety officer and a risk assessment carried out for the entire resource and its activities. Check statutory (and additional local) requirements for:

- Biological containment (e.g. suitable floors, benches etc. and any special considerations)
- Disposal of 'sharps', contaminated slides/cover-glasses, chemicals and biological material.
- User-supplied risk assessment documentation for all procedures they bring into the unit.
- Handling, storage and disposal of small volumes of solvents, e.g. for cleaning lenses etc.
- Air quality for comfortable working.
- Stress-free computer and microscope workstations.
- First aid provision (e.g. for 'sharps' incidents, emergency eye-wash etc.).
- Fire precautions (including access routes, emergency lighting etc.).
- Correct signs and indications of hazards (with details of unit or other responsible staff).

Resource support team

The team should consist of members with responsibility for:

- Overall scientific and technical strategy, public relations, fund raising etc. (the 'Director').
- Day to day operation and maintenance (the 'Manager'), assisted by support technicians.
- Scrutiny of potential, and on-going, projects and to offer advice (e.g. a 'science panel').
- User training (suitably qualified resource staff).

Small facilities may integrate the first two activities into a single position. The science panel can review projects to ensure appropriate use of resources, but it is inadvisable to attempt to run a busy resource by committee! Periodic reports from each individual maintain accountability but allows the resource to react quickly to changing needs. The manager can be the point of first contact with users and this post should include some individual research based on equipment or specific applications. This develops the available techniques and fosters an empathy with the research process.

User and project registration

Users should register each of their projects and instrument use. Basic details can be provided on a registration form that we recommend should include the following points:

- User and Supervisor contact details.
- A project name.
- A project manager (if other than the User or Supervisor).
- A work program outline.
- An estimate of resources required to complete the project.

If charging codes are based on research grants, users can have a separate login account for each project to ensure:

- Instrument problems can be directly related to the relevant users and applications.
- Charging can be automatically directed to normal account codes operating in a department.
- Special configurations or protocols can be made available to the relevant projects.

User training

Training users to conduct their own microscopy is preferred to 'service operation' where samples are handed to an operator for imaging. A skilled manager or technician may get through the samples more efficiently, but this breaks the vital link between the scientist and the experiment and can lead to errors. Researchers should understand how each instrument interacts with the sample and overall experiment. Dedicated microscopists often err towards 'better' images, rather than the most meaningful results.

Training should be progressive starting with an initial tutorial (about 1 hour). A shorter period of training at each of the early sessions will increase competence and reinforce skills. Progress should be monitored by the quality of meaningful data collected. Objective criteria for the quality of data required must be established for each project to avoid wasting time ever-improving images beyond what is required.

Users should be trained in data sampling to decide what images to collect, at what resolution, and with how many replications. A statistician on the scientific panel can resolve issues of significance.

It should be possible for a final year undergraduate to collect optical sections and z-series from an LSM after a one-hour tutorial. A preliminary session to assess the specimen, and on-hand support often mean that less formal training is needed. If longer is required, the initial training is probably too advanced! Sophisticated tasks can be learned gradually up to a level covering all the project needs.

Promotion of the unit and projects

As with all areas of science, dissemination of results (and also problems) is key to promoting competitive research. The resource and its projects will benefit from a range of seminar formats:

- Informal seminars covering practical microscopy aspects including trouble-shooting.
- Informal presentations of particular work in progress.
- Regular meetings of the science panel, perhaps also including discussion of major projects.
- One-day workshops focussing on specific techniques or applications areas.
- Formal training courses for beginners, advanced users, or for updating.
- Participation in (and/or organisation of) relevant microscopy conferences.

Meetings can be funded by equipment suppliers, microscopy-related societies and registration fees.

The best publicity is the dissemination of research results in peer-reviewed journals. Additional publications can promote the unit and particular areas of expertise (e.g. contributions to a departmental annual report or other handbook, reviews, press releases etc.) Microscopy and trade journals often run articles on particular techniques with manufacturer-sponsored pieces.

Bio-Rad produce user-information on applications and techniques in a variety of formats:

- Application notes
- Technical notes
- Confocal bibliography
- Multi-photon bibliography
- LSM Newsletters
- Other literature.

Microscopy web sites

'Web' and other Internet sites are rapidly becoming the standard way of bringing together user material, details of available techniques, topical results etc. Instrument booking can also be on-line with additional links to a range of other sites:

www.molecularprobes.com – Fluorescent probe data (inc. spectra)

www.chroma.com – Filter data (including .spectra)

microscopy.bio-rad.com – (including links to:)

- Interactive fluorochrome database
- Application notes
- Technical notes
- Bibliography
- Other microscopy and resource web pages.

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