# Setting Up and Running an Advanced Light Microscopy and Imaging Facility

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## ABSTRACT

During the last twenty years, interest in light microscopy and imaging techniques has grown in various fields, such as molecular and cellular biology, developmental biology, and neurobiology. In addition, the number of scientific articles and journals using these techniques is rapidly increasing. Nowadays, most research institutions require sophisticated microscopy systems to cover their investigation demands. In general, such instruments are too expensive and complex to be purchased and managed by a single laboratory or research group, so they have to be shared with other groups and supervised by specialized personnel. This is the reason why microscopy and imaging facilities are becoming so important at research institutions nowadays. In this unit, we have gathered and presented a number of issues and considerations from our own experience that we hope will be helpful when planning or setting up a new facility. *Curr. Protoc. Cytom.* 57:12.22.1-12.22.21. © 2011 by John Wiley & Sons, Inc.

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## INTRODUCTION

Light microscopy and digital imaging have undergone an unprecedented development during the last two decades. Cell biologists have benefited most from this progress and have also played a major role in pushing and orienting the evolution of this technology. As a result, light microscopes have evolved from small instruments located at the corner of laboratories to big optoelectronic systems, which are now installed in dedicated spaces of common access within research units. There are several reasons why light microscopy and imaging facilities are so extended nowadays. On one hand, the high cost of these instruments has forced researchers to acquire and use them as shared resources and, on the other hand, their complexity justifies the existence of qualified personnel that allows full access to entry-level users and, at the same time, maintains their optimal functional status (Cooke, 2000; Fernández-Suárez and Ting, 2008; Rae Chi, 2009; Combs, 2010; Ntziachristos, 2010; Smith et al., 2010; Spiller et al., 2010; Walter et al., 2010; Wessels et al., 2010). These facilities are becoming excellent platforms to promote scientific collaboration and resource exploitation at national and international levels.

In our experience, several aspects should be considered for the proper design of a facility. It is important to explicitly and precisely define the facility's purpose and functions, evaluate the number and type of potential users, the applications demanded, and the resources available, and ensure the long-term commitment of the scientific and management staff to the future development and sustainability of the facility. It is highly advisable to speak with potential users as much as possible through meetings and surveys and to write a preliminary report outlining the configuration, purpose, functions, funding, etc. of the projected facility. This draft should be revised and developed through discussions with the scientific and management staff. Frequent meetings with users and technicians of facilities already running would be highly beneficial during this stage.

Once a general agreement has been reached, it is time to consider the location and space needs, the staff requirements (e.g., number, qualification, aptitudes, and roles), the equipment to be purchased, etc. Additionally, it will be necessary to establish how the instrument's access will be managed, whether and how the use of the equipment and technical



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support will be charged for, and how training will be organized. In this regard, a good online booking and managing software platform becomes indispensable. We also stress the importance of an attractive, content-rich, and regularly updated Web site that serves as a meeting point for users and technical staff, as well as a repository of valuable information. Finally, it is essential that the technical staff feel like part of an active and important project. Involvement of the staff in decision-making, continuous training, and promotion are key elements to increase their motivation and performance.

Although the number of light microscopy and imaging facilities has increased considerably during the last years, there is not much information available for guidance through the setting up and running of a new one, apart from some interesting articles and book chapters (DeMaggio, 2002; Murphy, 2002; Humphrey, 2004; Anderson et al., 2007). In the present unit, we have looked back into our 10-year experience and we compiled what we consider to be the most important and useful tips to achieve this purpose.

## THE DESIGN: FIRST STEPS

Although different circumstances will surely occur at each institution, we believe that the first step in setting up a facility is to design a plan where all relevant aspects are included and detailed. This initial draft should be elaborated and discussed by a scientific committee containing a representation of potential users and the management staff. We advise that a member of the scientific staff, with the ability to recognize and integrate the different needs and interests of future users, take the responsibility to lead and coordinate the process.

The plan should cover the following aspects:

(1) *Identification of requirements*: number of potential users and applications to assess the instruments, technical staff, and space required.

(2) *Support* that the facility will provide, either purely technical and/or scientific advice or collaboration, and user training policy.

(3) *Resources* needed for the initial deployment (e.g., instruments, space conditioning, and technical staff) and subsequent periodic upgrades.

### Identification of Requirements

Before purchasing any equipment or recruiting technical personnel, the applications and instruments demanded by research groups should be clearly defined. The approach should be different when considering research institutions that are multidisciplinary or institutions that are sharply focused in one research area. In our experience, cell biologists, developmental biologists, neurobiologists, and immunologists are the most demanding users of time and instrument features. In such multidisciplinary scenarios, instrument versatility should be an important factor to consider. It is also important to identify future instrument and application needs and the number of users that might be interested. Surveys and meetings are useful to get proper feedback of scientific demands, as well as the day-to-day contact with scientists and their projects, which is probably the best way to identify future needs. Finally, a minimal space for the initial deployment and subsequent (2 to 3 years) growth of the facility should be defined. Optimal exploitation of the available space will depend on several parameters including its location, shape, compartmentalization, etc.

### Support

Facilities could be devised with different objectives and ideas in mind. However, in most cases, they can be cataloged as facilities aimed at instrument maintenance and user support (assistance and training), or as more advanced ones that put the emphasis in the development and setup of applications (or even instruments) through scientific collaboration. Most times, such advanced facilities are a logical evolution of the first ones. They are supported by a strong background and tradition in the field, generous funding, and are always run by highly qualified, motivated, and competent scientific and technical personnel.

In the first type of facility, the technical staff is involved in keeping the equipment running under optimal conditions and ensuring proper user assistance and training. This facility concept definitely saves a lot of time and trouble in the daily workload of scientists. Users just take care of their experimental designs and barely worry about the instruments. This approach is also very effective in preventing instrument failures. There are facilities that have taken this concept to its extreme and do not allow users to manipulate the instruments, which are exclusively operated by technicians. This saves time on training and ensures better instrument maintenance and user support, but it can easily lead to instrument saturation and reservation bottlenecks due to the limited availability of technicians and timetables.

This modality is only actually feasible at facilities with few instruments and few users. In the long run, the work of the technicians could become tedious and less motivating, leading to a loss of contact with investigation and information on new technologies and applications.

In the second type of facility, users discuss their technical challenges with the staff and together devise protocols and applications that are developed in collaboration, and thereafter made available to other users. Moreover, a facility with a number of active users and highly specialized technicians can be a perfect place for companies to develop and/or improve new applications or instruments. This also facilitates the access to new techniques and, at the same time, constitutes an additional funding source. Finally, this activity can be very helpful for technician training and motivation. However, additional technical staff would be needed to ensure proper standards of user support. In these facilities, a combination of people with diverse backgrounds (biologists, physicists, and software programmers) is highly advisable.

Another important consideration is which instruments and applications should be covered by a facility and which ones by the users' laboratories. In other words, where do we trace the line between basic microscopy applications in the laboratory and advanced instruments and applications at the facility? Cost (acquisition and maintenance) and complexity (use and repair) should be the parameters to consider. Other factors could be the size and funding of individual laboratories and how often they will need to use a particular instrument. Applications such as confocal and multi-photon microscopy, fluorescence life-time imaging microscopy (FLIM), total internal reflection fluorescence microscopy (TIRFM), high-content screening microscopy (HCS), cell microinjection or super-resolution microscopy are normally installed at facilities. On the other hand, stereomicroscopes, wide-field fluorescence microscopes, or even small compact confocal microscopy systems can be purchased by laboratories, and this decentralization would certainly help prevent saturation at the facility.

Frequently used reagents in microscopy applications, such as mounting media, fluorochromes, some primary antibodies, secondary antibodies, markers of cellular compartments, etc., could be purchased or even prepared at the facility and offered to users in small aliquots at a nominal price. This way, stocks can be better controlled and rapid replenishment will diminish reagent aging and outdating. Thus, depending on the number of users, the facility could maintain a battery of frequently used reagents that would ensure instant availability in the amounts that are needed. This means significant savings to individual laboratories and the chance to test them before purchase. At the same time, facilities can gather useful information from laboratories on recommended applications and conditions for the use of each reagent, to share it with other laboratories. Finally, scaling up purchase orders will certainly help to negotiate better deals with suppliers.

Facilities should also be able to give support on specialized software and digital imaging tools, but here we face a dilemma similar to the one posed when considering instrument purchase: which software should be owned by facilities and which one by laboratories? The same criteria, cost and complexity, should also determine the choice in this case. In our experience, it is highly desirable to actively encourage users to learn the necessary skills for basic imaging data management and to develop simple macros/plugins/journals, which would increase their throughput and proficiency in image processing and analysis. The facility staff should help the more reluctant users to overcome their fears with software applications and guide them through the first steps. However, users should understand that they need to invest time and effort in these tasks, since technicians' time is limited and the time they are available will never be enough to cover the specific requirements of every user.

## Resources

Availability of funds is always a critical factor in the design of a facility. Instrument acquisition and renewal, space conditioning, service maintenance contracts, and salaries of qualified personnel require a significant investment if the facility is committed to quality and competence. Funds can be obtained from public and private contests, either national or international, and also from enrollment in scientific networks or platforms set to develop microscopy technologies or to coordinate instrument and personnel resources. Such networks facilitate the access to high-end systems and promote the exchange of knowledge and expertise among technical and scientific staff from different institutions. "EuroBioImaging" (*http://www.eurobioimaging.eu*), the Australian Microscopy and Microanalysis

Research Facility (AMMRF) (*http://www.ammrf.org.au*), the Biomedical Informatics Research Network (*http://www.birncommunity.org*), the Center for Bioimage Informatics (*http://www.cbi.cmu.edu*), and other initiatives of the National Institutes of Health (*http://www.nih.gov*) are some projects in the right direction. Finally, funds might also come from agreements between research institutes and companies that join efforts to bring the academic and enterprise worlds closer, obtaining mutual benefits.

Funding opportunities for start-ups are usually easy to find. However, the importance of keeping a steady income flow year after year to maintain high-quality standards is frequently forgotten. Medium- and long-term policies should be planned from the beginning and a number of forecasts and decisions should be made early. For example, it is important to decide how the instrument's use will be charged for and whether the facility will receive some basic funding from the mother institution or if it will be exclusively supported by user charges. Additionally, depending on the financial model and the availability of similar equipment and applications offered in the surrounding area, it is important to decide if the facility will be opened to external users, from profit or nonprofit organizations, which might be a good source for additional funds. Agreements with companies for demonstrating and in-house testing instruments by potential buyers may again be useful for this purpose. These last two decisions will depend on the number of instruments available, the relative size of the internal and external user communities, and the number and qualifications of the technicians. If the facility has obtained a certification or accreditation from the International Organization for Standardization (ISO), a greater confidence will be projected on the services offered, thus raising the number of external users and income.

## EQUIPMENT

This section reviews some common considerations related to the acquisition of instruments in a facility. These have been split into two categories: systems and microscopes, and accessories and technician tools.

### Systems and Microscopes

Companies provide abundant information about the features and specifications of their microscopy and imaging products through their Web sites. Additionally, some information can also be obtained through any of the supplier's guides, which are published by journals and magazines such as Microscopy Today (*http://www.microscopy-today.com*), Microscopy and Analysis (*http://www.microscopy-analysis.com/light/supplier-directory*), OptoIQ (*http://buyersguide.lfw.optoiq.com/Search/index.html*), or Current Protocols (*http://cda.currentprotocols.com/WileyCDA/Section/id-380282.html*).

We stress the importance of taking into account the following considerations before making a final decision (see *UNIT 2.16*). First, a clear definition of the proposed use of the equipment should be done. This includes making a profile of expected users, their number, and type of imaging experiments involved. Although it may seem obvious, this exercise will help novice buyers narrow down the number and/or categories of instruments to be considered. Keep in mind that one instrument may be used for different types of experiments, and the same experiment could be carried out in different instruments. Therefore, the first task is to identify the type of experiments that will be performed by the majority of the users so that the appropriate category of instrument is selected.

Next, quality and performance should be compared among the selected instruments. This does not just mean creating thorough spreadsheets putting technical and performance specifications side-by-side. Each system has strengths and weaknesses. It really depends on what we expect. The best way to rate a system is to try it by ourselves using our own samples. Ask for similar instruments in other facilities or institutes, and go and try the instruments. Discuss and exchange views with experienced users. Companies should facilitate "hands-on" access to the systems that they are trying to sell.

It is not less important to take into account that instruments will be used by many users for several hours a day throughout the duration of years. We estimate that the typical life span of a microscopy system could be from 8 to 10 years. In a facility, instruments should be working optimally despite the stress associated with continuous use. Therefore, a key aspect to consider is the robustness and stability of the equipment. Whenever possible, go to other facilities where those systems have been

in use for some time and ask the following questions:

- Do they need frequent repairs?
- How long have they been out of service since their installation?
- Are they easily damaged by inexperienced hands?

Tightly linked to the latter point, the quality of the service and technical assistance of the provider should also be analyzed. In order to minimize the time that microscopes are out of order, service providers should have local technicians that can solve minor problems quickly. At the least, the diagnosis of the failure should be completed within 24 to 48 hr. More serious problems should be solved by more experienced external technicians in the shortest time possible. Although it is highly variable among countries, ask service providers questions like:

- How many technicians do they have in your area and how many systems do they give support to?
- How big is the area that they cover and where is the central office located?
- How many years of accumulated experience can they prove?
- If a more experienced outside technician were needed, how long would it take him to come?

Whenever possible, it is highly recommended to negotiate a technical maintenance service contract at the time of the purchase. Either the vendor or a third-party company should be able to offer different contract modalities and coverage levels (number of annual revisions, spare parts included or excluded, maximum response time, software upgrades, etc.). Cost and proven support efficiency of the service provider should be high on the list of purchase priorities.

There are other considerations to look into before purchase. For example, users highly value how easily instruments are operated without technical assistance; thus, intuitive controls for system operation at the hardware and software levels are a bonus. In addition, instrument design should facilitate basic maintenance and repair, so that the facility staff can solve most common problems, carry out programmed revisions, and change elements and accessories easily. It is convenient to depend as little as possible on external technicians so that instruments can run as long as possible.

A common dilemma when configuring systems for purchase is whether the instrument should be versatile and support a number of different applications or, on the contrary, if it should be focused and optimized for a particular application. Since companies implement techniques in a modular fashion, the temptation of populating one system with different applications is hard to resist. If versatility is favored, the system will benefit from a greater number of users; therefore, the money will have been better spent. However, the stress on the system will increase proportionately and also the probability of failure, affecting a higher number of users. On the other hand, if we choose to narrow the number of available applications, we will surely get increased tuning and performance, but the number of users will definitely decrease. Very often, available funds are not sufficient to acquire more complete configurations. It is then common to think that recently acquired equipment will be upgraded in the future. Unless the extra funds needed for upgrade arrive soon, we encourage purchasing "closed" systems optimally configured for specific purposes. New equipment and technologies develop very quickly and make projected upgrades obsolete and expensive. Furthermore, the equipment would probably need to be sent to the factory for the upgrade, which could leave it out of service for weeks or even months. Our advice here is to wait until more funds can be collected and get another system that can cover the missed applications and probably implement new ones. To summarize, the emphasis should be placed on the users and their current demands, rather than on instrument specifications and future upgrades. In a facility, the applications most demanded by users should be extensively covered by dedicated and customized systems.

Over time, facilities grow and the number of available instruments increases. It is common to find facilities where the majority of the instruments have been purchased from one of the major competing companies. Purchasing equipment from one company has some advantages. For example, users would not have to learn much to operate the different instruments since software design and hardware controls are very similar. In addition, some components and accessories can be exchanged, maintenance is easier for technicians, and service contracts can be better negotiated. However, as mentioned before, each system has strengths and weaknesses, so instruments from different companies may complement each other and offer a better coverage of different applications.

## Accessories and Technician Tools

Although most common image processing and analysis tasks are covered by open-source software, a facility might also consider purchasing specialized software packages for particular imaging tasks if a significant number of users are interested. Centralized acquisitions are often more thoroughly evaluated by technical staff experts who are in a better position to get better deals that include support, upgrades, and migration options. Commercial image processing and analysis programs offer better quality controls, experienced and customized support, and often a better integration with existing platforms and operating systems. However, we would advise that software developed in Open Source Software projects first be given a chance (Moore et al., 2008; Swedlow and Eliceiri, 2009; Linkert et al., 2010; O'Donoghue et al., 2010). In addition to the fact that they are free, these software packages are rapidly growing in features, power, and stability. Furthermore, an increasing community of scientists and programmers are continuously working to adapt these projects to the actual demands and challenges of current research (Table 12.22.1).

Depending on the experimental approaches, additional instruments could be needed at the facility. In our case, we have a 37°C, $CO_2$ incubator, a vertical laminar air flow hood, a refrigerator, and a −20°C freezer. Other common accessories are: fluorescence and halogen lamps, objectives, filters, immersion oil, etc. for microscopes; incubation chambers and peristaltic and/or syringe pumps for in vivo imaging; plasticware or culture media for cell culture; slides, coverslips, or mounting media for immunofluorescence; and regular office supplies (Table 12.22.2).

Finally, some additional tools can be recommended for maintenance and cleaning. For example, a laser power meter is very useful to check laser alignment and performance and to detect optical path problems. Other consumables include cleaning tools, such as wipes, air sprayers, cleaning solutions, and cotton tips and calibration samples, such as grid-scaled and mirror slides, beads (ring, multicolor), and fluorescence calibration standards.

## Location and Distribution

The space within a facility is commonly divided into dark rooms dedicated to microscopes, some areas for digital imaging workstations, some bench space to facilitate basic experimental manipulations, space for cell culture and preparation of live samples including a $CO_2$ incubator and a cell culture hood space for a fridge and freezers, and an office.

Advanced microscopy systems are especially sensitive to vibrations, electrical interferences, drafts, and temperature changes. Thus, facilities located close to elevators, centrifuges, compressors (from cold rooms, for example), freezers, refrigerators, or microwave sources should be avoided. The basement and lower floors are usually less prone to vibrations and should be preferred. It is also important to evaluate floor loading and resistance capacity, particularly in rooms where heavy antivibration tables will be placed.

Another issue to consider is the quality of the electrical power supply, which may affect scanning, laser stability, and image acquisition. It should be free of electromagnetic spikes, noise, and frequency instability. Ideally, the power supply line that feeds the facility should come directly from the central distribution panel of the building in an attempt to isolate it from interferences coming from neighbor laboratories. An independent and dedicated ground connection for the facility will help to minimize electrical noise. We also encourage having well identified electrical distribution panels close to the facility, which can be easily managed by technicians. Finally, instruments that generate vibrations may also produce electrical interferences. Try to set up the facility far from these instruments or vice versa.

It is also very important to prevent instrument damage caused by power failures. Laser boxes become very hot during normal operation and need constant refrigeration. In case of a power failure, the possibility of laser units being damaged by excessive heat is high. Therefore, it is a good idea to consider using uninterruptible power supply (UPS) systems that, in case of blackout, will maintain the systems working long enough for proper shutdown and cooling. These units not only protect from power failures, but they also isolate the systems from electrical and magnetic fluctuations in normal operation, thereby improving power stability.

The distribution of the space may have different configurations. The first major division separated by brick walls should define an area of dark rooms dedicated to microscope systems and an area dedicated to bench, office, etc., which could have natural light. An optimal starting point to configure the instrument dark rooms is to have an open space that can

**Table 12.22.1** Free Open Source Software

| General tools | 3D and rendering tools | Other specific tools | File management |
|---|---|---|---|
| BioImageXD (*http://www.bioimagexd.net*)<br>FARSIGHT (*http://www.farsight-toolkit.org*)<br>Fiji (*http://pacific.mpi-cbg.de*)<br>ImageJ (*http://rsbweb.nih.gov/ij*)<br>ImageTrack (*http://www.ucalgary.ca/styslab/imagetrak*)<br>ImageTool (*http://ddsdx.uthscsa.edu/dig*)<br>Micromanager (*http://www.micro-manager.org*)<br>Priism/IVE (*http://msg.ucsf.edu/IVE/index.html*)<br>V3D, VANO and Cell Explorer (*http://penglab.janelia.org/software/*) | Blender (*http://www.blender.org*)<br>IMOD (*http://bio3d.colorado.edu/imod*)<br>ImageSurfer (*http://imagesurfer.cs.unc.edu*)<br>ITK (*http://www.itk.org*)<br>MIPAV (*http://mipav.cit.nih.gov*)<br>Osirix (*http://www.osirix-viewer.com*)<br>POV Ray (*http://www.povray.org*)<br>3DSlicer (*http://www.slicer.org*)<br>Visual3D (*http://software.ronhoebe.com/free*)<br>VOXX (*http://www.nephrology.iupui.edu/imaging/voxx*)<br>VTK (*http://www.vtk.org*) | CellProfiler (*http://www.cellprofiler.org*)<br>Cirklo (*http://calendar.igc.gulbenkian.pt/cirklo*)<br>VirtualCell (*http://vcell.org*) | Bio-Formats (*http://www.loci.wisc.edu*)<br>Bisque (*http://www.bioimage.ucsb.edu*)<br>MIPA (*http://www.serverko.sk/mipa*)<br>OME-OMERO (*http://www.openmicroscopy.org*)<br>VisBio (*http://tiny.cc/TOZad*) |

**Table 12.22.2** Microscopy Resources

| Cell incubation chambers | Peristaltic and syringe pumps | Fluorescence and halogen lamps | Slides, coverslips, and culture dishes | Filters | Fluorochrome databases |
|---|---|---|---|---|---|
| Life Imaging Services (*http://www.lis.ch*)<br>Warner Instruments (*http://www.warneronline.com*)<br>Pecon-Lacon (*http://www.pecon.biz*)<br>Bioptechs (*http://www.bioptechs.com*)<br>Cell Kinetics (*http://www.cellkinetics.com*)<br>Nikon MicroscopyU (*http://www.microscopyu.com/articles/livecellimaging/chamberresources.html*) | Gilson (*http://www.gilson.com*)<br>Harvard Apparatus (*http://www.harvardapparatus.com*)<br>Warner Instruments (*http://www.warneronline.com*)<br>GE Healthcare (*http://www.gehealthcare.com*)<br>Ismatec (*http://www.ismatec.com*)<br>Major Science (*http://www.major-sci.com*)<br>Millipore (*http://www.millipore.com*)<br>IITC Lifesciences (*http://www.iitcinc.com*)<br>myNeurolab (*http://www.myneurolab.com*)<br>Stoelting (*http://www.stoeltingco.com*)<br>World Precision Instruments (*http://www.wpiinc.com*) | Philips (*http://www.lighting.philips.com*)<br>Osram (*http://www.osram.com*)<br>Ushio (*http://www.ushio.com*) | BarloWorld Scientific (*http://www.barloworld-scientific.com*)<br>Becton Dickinson (*http://www.bdbeurope.com*)<br>BellCo Glass (*http://www.bellcoglass.com*)<br>Bioscience Tools (*http://biosciencetools.com*)<br>Cytoo (*http://www.cytoo.com*)<br>Fisher Scientific (*http://www.fishersci.com*)<br>Grace Biolabs (*http://www.gracebio.com*)<br>Greiner Bio One (*http://www.greinerbioone.com*)<br>Integrated BioDiagnostics (*http://www.ibidi.com*)<br>Invitrogen (*http://www.invitrogen.com*)<br>Knittel Glaeser (*http://www.knittel-glaeser.de*)<br>MatTek (*http://www.glass-bottom-dishes.com*)<br>Menzel Gläser (*http://www.menzel.de*)<br>Neuroprobe (*http://www.neuroprobe.com*)<br>SPL (*http://www.ispl.co.kr*)<br>Thermo Scientific (*http://www.nuncbrand.com*)<br>Willco Wells (*http://www.willcowells.com*)<br>World Precision Instruments (*http://www.wpiinc.com*) | Chroma (*http://www.chroma.com*)<br>Omega (*http://www.omegafilters.com*)<br>Semrock (*http://www.semrock.com*)<br>Melles Griot (*http://www.cvimellesgriot.com*)<br>AHF (*http://www.ahf.de*) | Mol. Cell. Biol. Dept. (Arizona U.) (*http://www.mcb.arizona.edu/IPC/spectra_page.htm*)<br>PubSpectra (*http://home.earthlink.net/~pubspectra*)<br>Fluorophores.org (*http://www.fluorophores.tugraz.at*)<br>Yale Genetics (*http://info.med.yale.edu/genetics/ward/tavi/FISHdyes2.html*)<br>Zeiss (*http://www.micro-shop.zeiss.com*)<br>Olympus (*http://www.olympusconfocal.com/java/dualprobes/index.html*)<br>On-line Spectra Viewer (*http://www.online-spectra.com*)<br>Curv-O-Matic (Omega) (*http://www.omegafilters.com/curvo2/index.php?*)<br>Invitrogen (*http://www.invitrogen.com/site/us/en/home/support/Research-Tools/Fluorescence-SpectraViewer.html*)<br>Becton Dickinson (*http://www.bdbiosciences.com/research/multicolor/tools/index.jsp*)<br>PhotochemCAD (*http://www.photochemcad.com*) |

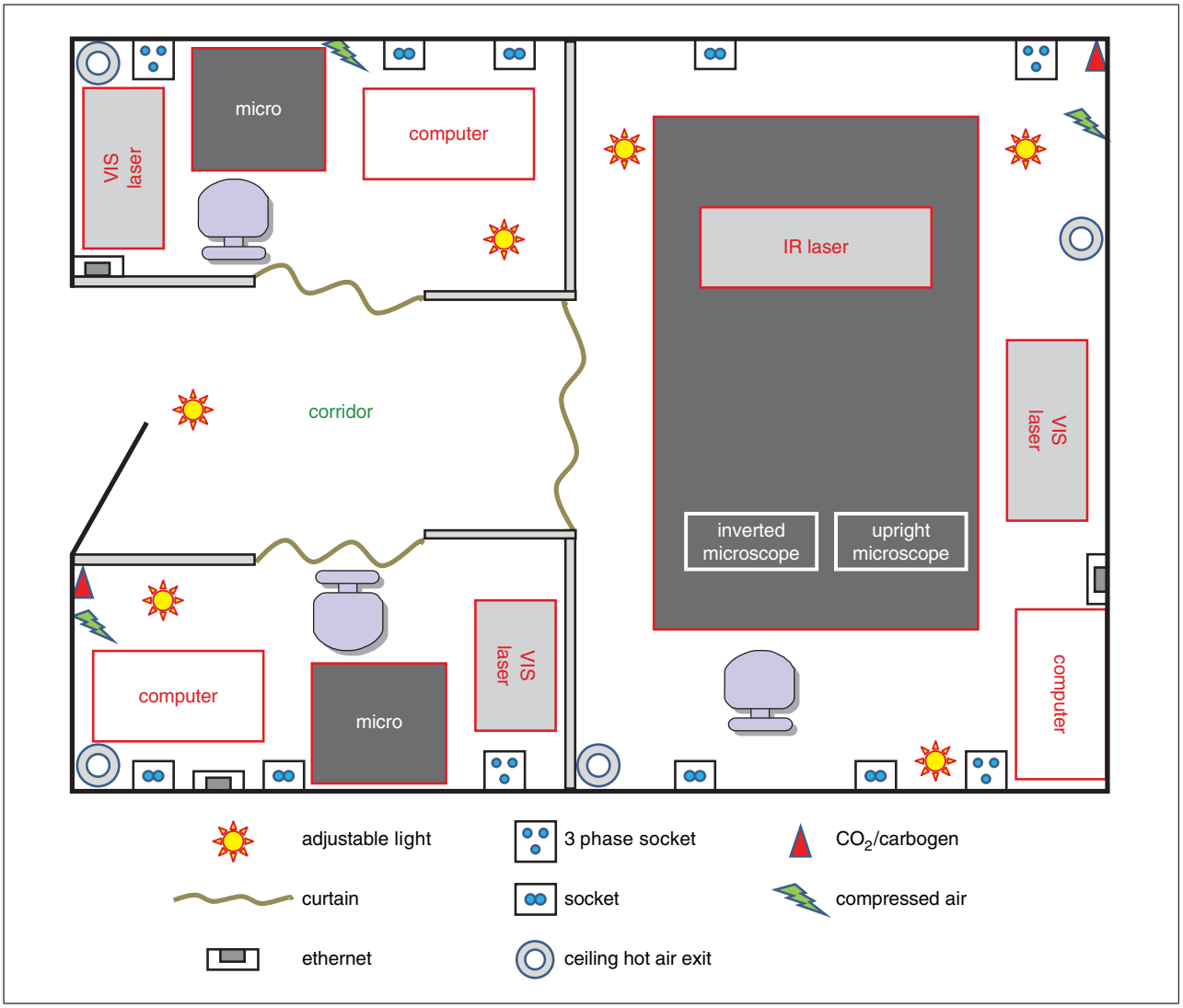


**Figure 12.22.1** Distribution of instruments. This is the distribution of one multiphoton and two confocal systems in one of the author's facility rooms. Each room accommodates one instrument and is isolated from the corridor by screens and black sliding curtains. They should have space for microscopes (Micro), visible (VIS) and infrared (IR) laser racks, computers, and some comfortable chairs. Adjustable lights are recommended. Additionally, $CO_2$/carbogen, compressed air and hot air exits, as well as ethernet and electrical sockets might be needed.

be freely split into smaller rooms separated by fixed opaque screens according to the number of instruments and the space needed by each one. Access to the dark area could be granted through special round doors that minimize light fluctuations and airstreams. Each dark room would accommodate one or two instruments and would be connected to a distribution corridor through black sliding curtains (Fig. 12.22.1). The dark room dividers can be fixed to the floor and walls but should not reach the ceiling, so that an open space (at least 50 cm from the ceiling) can be shared to allow air circulation among the rooms and reduce temperature fluctuations.

Avoiding temperature and humidity fluctuations is also critical for good performance of the microscopy equipment. Temperature should not exceed 26°C and humidity 60% to 65% (though this may depend on local climate) in the dark rooms, but even more important is to keep temperature and humidity as stable as possible, avoiding oscillations. Ceiling-mounted split air conditioning installed at the distribution corridors of the dark areas ensures better air circulation and keeps airflow away from the microscopes. It would be better if they could be regulated independently of the building's general cooling system. Check air conditioner filters regularly to remove dust and ensure effective cooling. Whenever possible, a good option is to take the laser's refrigeration systems out of the dark rooms to decrease noise, vibration, and heat. Alternatively,

15- to 20-cm wide tubing should be installed over the heat generation units and coupled to extraction fans to pump hot air out of the room. Such fans should be placed as far away as possible from the instruments, either outside of the building (e.g., roof, etc.) or in dedicated ventilation chimneys.

Fluorescence microscopy requires very dim ambient light (close to darkness). Thus, it is highly recommended to have an illumination system whose intensity can be gradually and independently regulated in each room, providing uniform and soft illumination. Walls should be painted matte in dark tones.

Other installations that should be planned in advance are $CO_2$ and/or carbogen lines for incubation chambers and in vivo experiments, compressed air supply for antivibration tables, 15- to 20-cm round holes in the walls or ceiling for hot air extraction, and a generous distribution of power and ethernet sockets located at the walls, around 110 cm from the floor (Fig. 12.22.1).

Finally, it should be taken into account that users must spend hours in dark rooms sitting at the microscope in front of a computer monitor. Therefore, it is crucial to properly choose the location and distribution of the instruments to create a comfortable and safe working environment. Handy chairs, an appropriate location of the computer monitor, keyboard and mouse, sufficient table space to make annotations, etc. are important details that reduce the fatigue associated with routine work.

## SPECIALIZED STAFF

In a typical facility, specialized technicians operate and maintain the microscopy and digital imaging equipment and train and assist scientists to get the best possible results from their experiments. Additionally, they deal with the general management of the facility (invoicing, budgeting, reports, basic regulations, etc.), maintain the Web page (on-line reservations, handy information for users, etc.), have direct contact with users (complaints, surveys, suggestions, etc.), participate in seminars and courses, and perform other tasks, such as stock maintenance, contact with companies' representatives, etc.

It is important to define the background, qualities, and experience that aspirants to such technical positions should have. In addition to good theoretical and practical knowledge on microscopy and imaging, a background in biology, physics and/or computer science, and an acceptable level of spoken and written English, are recommended. The aspirant should have the ability to organize and become responsible for the work, good teaching and interpersonal communication aptitudes, skill and care with delicate parts (e.g., lenses, etc.) of the instruments and, no less important, initiative and the ability to solve problems.

Light microscopy and imaging are expanding rapidly. Therefore, technical personnel should be continuously trained and stimulated according to their competence and responsibility. Creating a team of experienced technical staff requires time. It is good advice to encourage the technical personnel to attend courses and workshops, and to visit other laboratories (Table 12.22.3). These activities not only contribute to their training, but also allow them to network with other professionals and share experiences. On the other hand, technical staff should ideally work as a team. Namely, everyone should be able to do the job of others (though some kind of specialization is inevitable) and should feel that his/her contribution is equally important and necessary. In this sense, an intelligent boss not only should correct mistakes but also appreciate and emphasize right decisions and initiatives. A good working environment usually leads to better competence.

Although not strictly related to the technical staff, we think that the involvement of one or more members of the scientific staff in supervising the facility can be helpful. Such scientists should be knowledgeable and interested in the fields of microscopy and imaging and, if possible, their research should involve the use or development of light microscopy and imaging techniques. As members of the scientific staff, they could have better knowledge of the needs of other research groups and therefore constitute an operative link among the technical, scientific, and management staff. They are in a privileged position to "sense" and anticipate the needs (equipment, space, personnel and organization) and to plan the future orientation (new applications and technologies, projects and purchases, etc.) of the facility.

## MANAGEMENT

### Facility Fee

Once the setup of the facility has been completed, it is time to review the major points associated with its maintenance and future growth. In the past, it was common to find research support services offering access to big instrument-based applications either for free or at a nominal fee, which only covered

**Table 12.22.3** Training and Information

| Courses and workshops | Scientific journals | Books and "online" resources | Specialized magazines | Societies | Forums |
|---|---|---|---|---|---|
| Advanced Microscopy - Live (*http://www.lifesci.ucsb.edu/mcdb*) | Annual Reviews (*http://www.annualreviews.org*) | Andor Learning Center (*http://www.andor.com/learning*) | Advance Imaging (*http://www.advancedimagingpro.com*) | American Microscopical Society (*http://amicros.org*) | Academia.edu (*http://www.academia.edu*) |
| Annual Live Cell Microscopy Course (*http://www.3dcourse.ubc.ca*) | Bioimaging (*http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1361-6374*) | Basic Confocal Microscopy. Bob Price and Jay Jerome | Imaging and Microscopy (*http://www.imaging-git.com/*) | American Society for Biochemistry and Molecular Biology (*http://www.asbmb.org*) | ImageJ (*http://rsb.info.nih.gov/ij/list.html*) |
| Microscopy in Oeiras (*http://uic.igc.gulbenkian.pt/microscopy.htm*) | Biomedical Engineering Online (*http://www.biomedical-engineering-online.com*) | Basic Methods in Microscopy: Protocols and Concepts from Cells: A Laboratory Manual. D. Spector and R. Goldman | Microscopy and Analysis (*http://www.microscopy-analysis.com*) | American Society for Cell Biology (*http://www.ascb.org*) | Listserver Buffalo U. (*http://listserv.buffalo.edu*) |
| Workshop on FRET Microscopy (*http://www.kcci.virginia.edu/workshop/index.php*) | Biophysical Journal (*http://www.cell.com/biophysj*) | Confocal Microscopy for Biologists. A. R. Hibbs | Microscopy and Microanalysis (*http://journals.cambridge.org/action/displayJournal?jid=MAM*) | American Society for Microbiology (*http://www.asm.org*) | Listserver Minnesota U. (*http://lists.umn.edu/cgi-bin/wa?INDEX*) |
| BioImaging UK (*http://www.bioimaginguk.org*) | Biotechniques (*http://www.biotechniques.com*) | Confocal and Two-photon Microscopy. Foundations, Applications and Advances. A. Diaspro | Microscopy today (*http://www.microscopy-today.com*) | Association of Biomolecular Resource Facilities (*http://www.abrf.org*) | Microscopy Listserver (*http://microscopy.com*) |
| CSHL (*http://meetings.cshl.org*) | CSHL Protocols (*http://cshprotocols.cshlp.org*) | Confocal Microscopy. Methods and Protocols. St. W. Paddock | Optics InfoBase (*http://www.opticsinfobase.org*) | Australian Microscopy and Microanalysis Society (*http://www.microscopy.org.au*) | Molecular Expressions (*http://micro.magnet.fsu.edu*) |
| ELMI (*http://www.embl.org/elmi*) | Current Protocols (*http://www.currentprotocols.com*) | Confocal Microscopy. B. Matsumoto | OptoIQ (*http://www.optoiq.com*) | Australian Optical Society (*http://aos.physics.mq.edu.au*) | |
| EMBL (*http://www.embl.de*) | Cytometry (*http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1552-4930*) | Digital Microscopy. Third edition. Methods in Cell Biology, Volume 81. Greenfield Sluder and David E. Wolf | Photonics (*http://www.photonics.com*) | Biophysical Society (*http://www.biophysics.org*) | |
| EMBO (*http://www.embo.org*) | European Biophysical Journal (*http://www.springerlink.com/content/100412*) | Fluorescence Applications in Biotechnology and the Life Sciences. Ewa M. Goldys Fluorescence Microscopy. B. Herman | The Imaging Source (*http://www.theimagingsource.com*) | European Biophysical Societies' Association (*http://www.ebsa.org*) | |
| EMS (*http://www.eurmicsoc.org*) | | | Lightwave (*http://www.lightwaveonline.com*) | European Light Microscopy Initiative (*http://www.embl.org/elmi/*) | |
| FEBS (*http://www.febs.org*) | | | | | |
| Focus on Microscopy (*http://www.focusonmicroscopy.org*) | | | | | |
| Fluorescence Foundation (*http://www.fluorescence-foundation.org*) | | | | | |

*continued*

**Table 12.22.3** Training and Information, *continued*

| Courses and workshops | Scientific journals | Books and "online" resources | Specialized magazines | Societies | Forums |
|---|---|---|---|---|---|
| ImageJ Conference (*http://imagejconf.tudor.lu*)<br>Italian Physical Society (*http://www.sif.it*)<br>Marine Biological Laboratory (*http://www.mbl.edu*)<br>MSA (*http://www.microscopy.org*)<br>Olympus Fluoview (*http://www.olympusfluoview.com/resources/courses.html*)<br>RMS (*http://www.rms.org.uk*)<br>SPIE (*http://spie.org*) | Histochemistry and Cell Biology (*http://www.springerlink.com/content/100427*)<br>Journal of Biological Physics (*http://www.springerlink.com/content/0092-0606*)<br>Journal of Biomolecular Techniques (*http://jbt.abrf.org*)<br>Journal of Digital Imaging (*http://www.scarnet.org*)<br>Journal of Fluorescence (*http://www.springerlink.com/content/104905*)<br>Journal of Microscopy (*http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1365-2818*)<br>Journal of Histochemistry and Cytochemistry (*http://www.jhc.org*)<br>Journal of Optics (*http://iopscience.iop.org*)<br>Journal of Visualized Experiments (*http://www.jove.com*)<br>Methods in Cell Biology (*http://www.sciencedirect.com/science/journal/0091679X*)<br>Methods in Enzymology (*http://www.sciencedirect.com/science/bookseries/00766879*)<br>Micron (*http://www.sciencedirect.com/science/journal/09684328*)<br>Microscopy Research and Technique (*http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1097-0029*)<br>Nature (*http://www.nature.com*)<br>PLoS ONE (*http://www.plosone.org*)<br>Science (*http://www.sciencemag.org*) | Fluorescent and luminescent probes for biological activity. W.T. Watson<br>Fundamentals of Light Microscopy and Electronic Imaging. Douglas B. Murphy<br>Hamatsu Learning Center (*http://learn.hamamatsu.com*)<br>Handbook of Biological Confocal Microscopy. J. Pawley<br>Immunocytochemical Methods and Protocols. L. C. Javois<br>Immunocytochemistry: A practical Guide for Biomedical research. Richard W. Burry<br>Life Cell Imaging. Methods and Protocols. Dimitri B. Papkovsky<br>Light and video microscopy. Randy O. Wayne<br>Light Microscopy in Biology. A Practical Approach. Alan J. Lacey<br>Methods in Cell Biology Vol. 70. Cell Biological Applications of Imaging Living Cells. R. Rizzuto and C. Fasolato<br>MicroscopyU (*http://www.microscopyu.com*)<br>Microscopy Resource Center (*http://www.olympusmicro.com*)<br>Molecular Expressions (*http://micro.magnet.fsu.edu*)<br>Optical Imaging Techniques in Cell Biology. Guy Cox<br>Principles of Fluorescence Spectroscoy. Joseph R. Lakowicz<br>Photometrics Learning Zone (*http://www.photometrics.com/resources*)<br>Protein Localization by Fluorescence Microscopy. W. J. Allan<br>Protein Localization by Fluorescence Microscopy. A Practical Approach. Viki Allan.<br>The Cell. An imaging library (*http://www.cellimagelibrary.org*)<br>Zeiss Campus (*http://zeiss-campus.magnet.fsu.edu*) | | Euro-BioImaging (*http://www.eurobioimaging.eu*)<br>European Microscopy Society (*http://www.eurmicsoc.org*)<br>FASEB (*http://www.faseb.org*)<br>FEBS (*http://www.febs.org*)<br>Federation of European Neuroscience Societies (*http://www.fens.org*)<br>Fluorescence Foundation (*http://www.fluorescence-foundation.org*)<br>International Society for Stereology (*http://www.stereologysociety.org*)<br>International Federation of Societies for Microscopy (*http://www.ifsm.uconn.edu*)<br>Japanese Society of Microscopy (*http://www.microscopy.or.jp*)<br>Microscopy Society of America (*http://www.microscopy.org*)<br>Optical Society of America (*http://www.osa.org*)<br>Optical Society of Japan (*http://annex.jsap.or.jp/OSJ*)<br>Postal Microscopical Society (*http://myweb.tiscali.co.uk/postalmicsoc*)<br>Royal Microscopical Society (*http://www.rms.org.uk*)<br>Spanish Society for Cell Biology (*http://www.sebc.es*)<br>Spanish Society for Biophysics (*http://www.sbe.es*)<br>Spanish Society for Microscopy (*http://www.microscopia.org*)<br>Spanish Society for Neuroscience (*http://www.websenc.es*)<br>Society for Neuroscience (*http://web.sfn.org*) | |

the consumables. This is not the case anymore. Current facilities charge for the use of their instruments to finance a substantial part (or even all) of its budget. Of course, each institution has its own policy in this respect and the percentage of the running expenses passed on to the users may vary. One obvious argument to establish a fee-based system is to invest in new equipment. Although charging fees is never welcomed by researchers, it makes them more responsible and realistic when planning experiments and booking instruments. At the same time, they get a more accurate perception of the cost and maintenance of instruments, which is associated with a greater care and concern. Finally, the necessary accounting work related to invoicing facilitates the tasks involved in recording usage statistics, such as equipment workload, rush hours, breakdowns, etc.

To configure a fee schedule we may take into account the initial cost of the instrument, repairs (parts and labor), maintenance service contract, consumables (lamps, lasers, immersion oil, etc.), and salaries of the technical staff. It is useful to make a realistic estimation of the life span of each instrument (in years) and its workload (i.e., hours per week) to obtain an approximate amortized cost (per hour). Rates should be established for each instrument independently.

However, the elaboration of a fee schedule can get more complex if we consider additional parameters. For example, we may want to distinguish different categories of users, such as internal and external ones and users that work at profit or nonprofit institutions. An appropriate balance should be found between high rates, which may discourage the use of the facility, and low rates, which might attract so many external users that local ones could be affected in their daily work. The circumstances affecting this balance may vary greatly among facilities at different research institutions. In any case, we believe that facilities should be open to as many users as possible. After all, this is why they were built. Opening access to users of other institutions within the campus and local areas will increase the choices and alternatives of advanced microscopy and digital imaging applications in the area, and it may also promote scientific collaboration.

Another consideration involves deciding whether the mother institution should subsidize the facility and to what extent. The contribution could be a fixed amount negotiated every year or a percentage of the annual expenses. Users will benefit from this extra income since their billing will be reduced. Thus, facility subsidizing relies on the institutional policy, which could decide whether to contribute to its startup and consolidation during the first years or even to stably promote the use of advanced light microscopy applications.

In our facility, we distinguish between occasional and regular users. Rates are reduced proportionally according to the use of the equipment, which could be applied for each instrument or not. So, research groups will benefit from lower prices if they accumulate more than a given amount of hours or charge per week or month. This policy can compensate for the higher contribution of regular users to the maintenance of the facility. Several rate-reduction steps could be established. Our advice is to start with a simple system with no more than two or three different rates, which could be modified later based on the results.

Other considerations might be included to further customize the fee schedule. For example, an additional differentiation could be established between novice and experienced users. Novice users need technical assistance to operate the instruments, so an extra charge might be added in these cases. Finally, one way to improve user responsibility and solidarity could be to establish special rates for persistently negligent users.

### *Control and regulation*

Unfortunately, user demand is often high and facility resources are often limited. In such cases, there is no alternative but to establish some rules to ensure ordered access to instruments and to accommodate as many users as possible. Rules are usually established when a problem is detected or when they are suggested by users. Unfortunately, it is not uncommon to find that a minority of users tend to be selfish and careless. In these cases, rules become essential to prolong the life span of instruments and to make them more productive.

We suggest the following common sense rules:

- Sometimes experiments do not progress as planned and instrument reservations made in advance cannot be used. In such cases, freeing the reserved time as soon as possible, with a notification to other potential users, is highly recommended. It is also a good habit to have each user notify the next one in the reservation list when finished using the instrument, so that "dead" times can be reduced.

• Restrict or ban "preventive" reservations, that is to say, reservations made, e.g, more than 15 days in advance. In our experience, such reservations have a higher probability of remaining unused or being released too late to be exploited by other users.

• Whenever possible, users should distribute their work among different instruments. Establishment of some "preferred" systems for basic and common techniques leads to underutilization of other equally capable instruments and to decreased service output. If there is a "feeling" that expected results are not equally obtained with different instruments, users should ask the technical staff for help.

• Try to perform long-term in vivo experiments during nights or weekends.

Occasionally, these common sense rules are not sufficient and some extra limits may become necessary, such as:

• Set the maximum duration for a single reservation (e.g., 3 hr).

• Set the maximum weekly hours that a user can work in a system (e.g., 8 to 10 hr).

• In our facility, we have set "high-demand" and "low-demand" hours during the day and established different rules for each time slot. For example, during high-demand hours, the maximum length of a single reservation is reduced to 2 hr and the weekly limit of accumulated user time per instrument goes down to 6.

Reservations not used or cancelled with ample notice (e.g., 2 hr in advance) could also be invoiced.

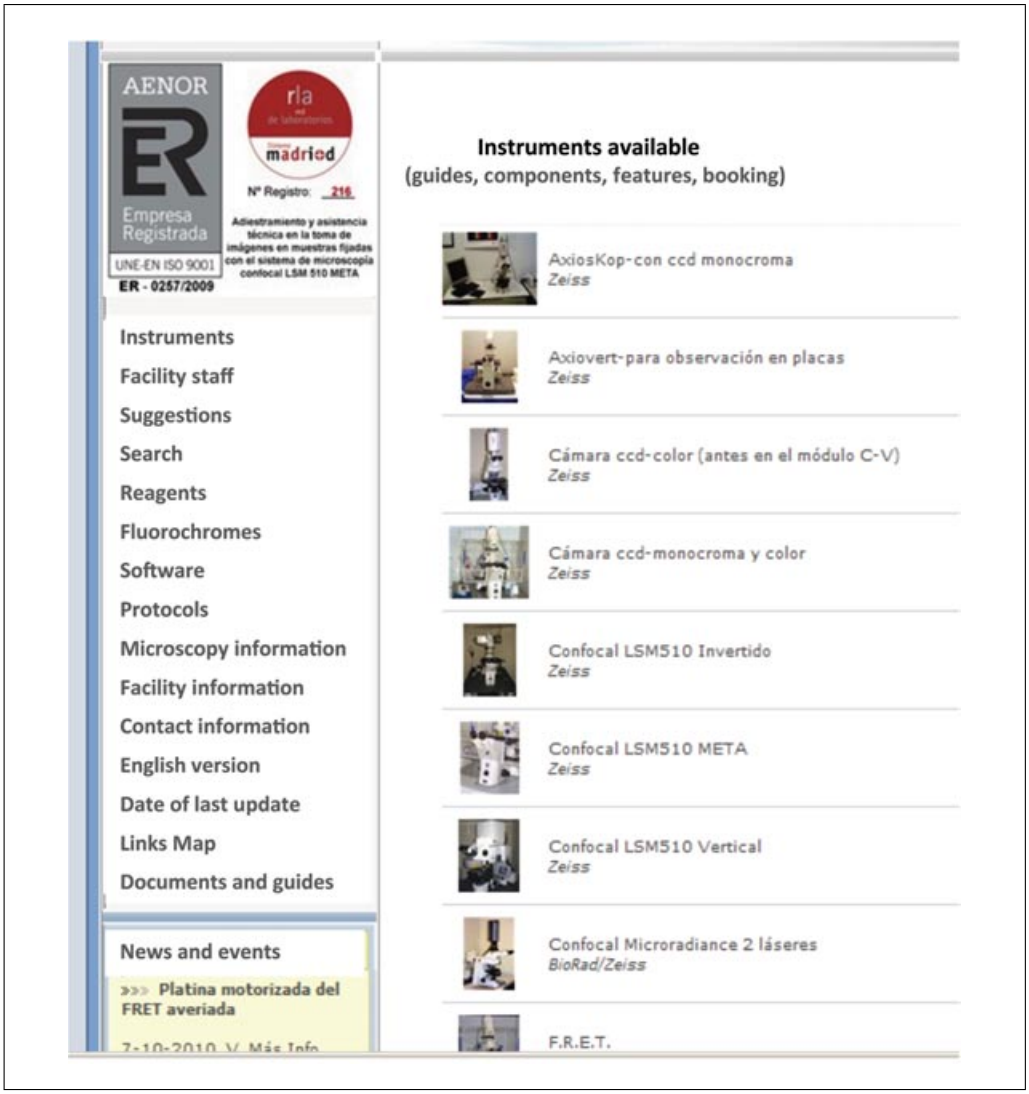


**Figure 12.22.2** A good and practical Web site, including a Web-based equipment scheduling database, is very helpful for the facility performance. In ours, we include information about microscopy, imaging, protocols and reagents; information about events (workshops, seminars, courses, etc.) and news; and information about the facility (available equipment, guides, article references, useful links, booking, etc.).

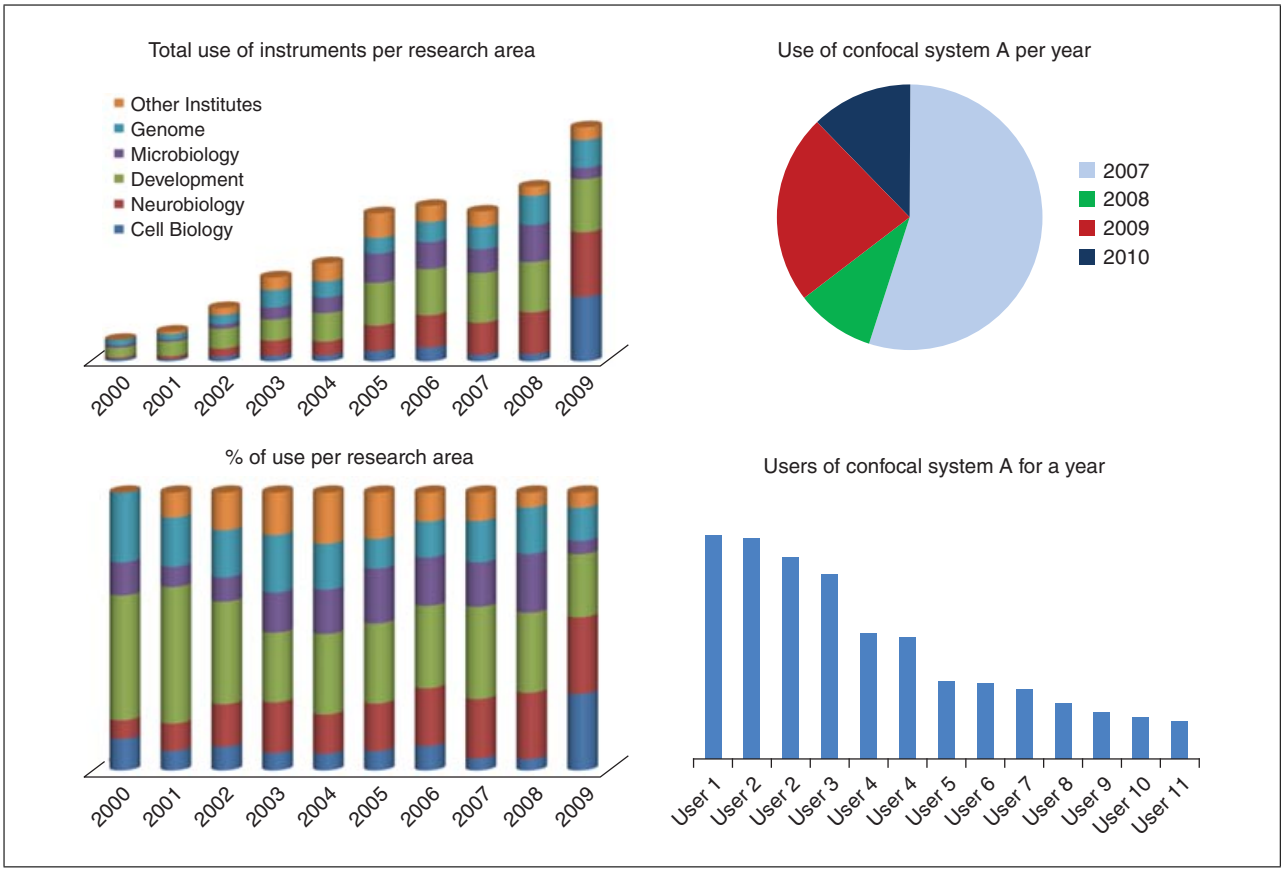


**Figure 12.22.3** Facility statistics. A complete register of the instrument's use allows recovering very valuable information to improve the facility. Here, we show some examples: use of instruments per research areas, per years, and per laboratories/users. For the color version of this figure go to *http://www.currentprotocols.com/protocol/cy1222*.

• Access control to the facility, by means of user identification cards and PINs, is recommended. It allows keeping a register of the people entering the facility and deters robbery and vandalism.

### *Web-based management*

In a modern facility, a well-designed, content-rich and updated Web site with its associated applications is key to reaching high levels of competence and efficiency. The client-server concept of Web-based applications enormously facilitates and automates routine tasks, such as online reservations, invoicing, etc. In addition, the facility Web site is an excellent communication channel among users and technicians and can be used as a repository of useful information about available instruments, protocols, products, guides, events, and news (Tables 12.22.2 and 12.22.3). Finally, a well-designed Web site enhances the external visibility of both the facility and the institution.

A good way to know what information should be present in the Web site is to listen to and note all user's questions and suggestions. In this sense, the Web site could be considered as a complete collection of easily accessible frequently asked questions (FAQs) (Fig. 12.22.2).

The instrument-scheduling database is probably the most useful Web tool for the management of the facility. Online reservations, invoicing, usage reports, and statistics are usually processed and stored on a main server. Selecting the appropriate scheduling and database software to manage all these data is essential for daily operation. The application should be able to manage "online" booking, allowing registered users to reserve instruments anytime and anywhere, notify whether they need assistance, and report incidents. It should also allow the administrator to set the established rules and limits, and automatically warn and prevent reservations exceeding established limits from being completed. It should keep a detailed record of each instrument (e.g., users, incidences, repairs, etc.) and generate usage reports and statistics, which are very useful in order to get a general outlook of the facility performance and to identify future requirements (Fig. 12.22.3).

Finally, it is highly desirable that the application can manage detailed user invoicing, which should include instruments, user names, dates, hours, etc., and be able to apply the established pricing rules to each user automatically. This saves a lot of management work time. A certification or accreditation from the International Organization for Standardization (ISO) can also help to systematize all this information. In our experience, the "Pasteur/Rockefeller Platform Management System" (*https://ppms.info*) developed at the Pasteur Institute provides many of the tools needed for scheduling, invoicing, and record keeping. Nonprofit and educational Institutions can use it after signing a license agreement. Other Web-based applications include Calcium (*http://www.brownbearsw.com/calcium*), Cirklo (*http://calendar.igc.gulbenkian.pt/cirklo*), Connect Daily (*http://www.mhsoftware.com*), E Lab Experts (*http://www.elabexperts.com*), General Resource Management (*http://www.grm.at*), OnCore Scheduler (*http://demo.arl.arizona.edu*), and PhpScheduleIt (*http://php.brickhost.com*).

### *Computers and file storage*

Tasks associated with computer maintenance, file handling, operating systems security, and user restrictions (e.g., user privileges, file permissions, events register, folder options, network sharing, etc.) should be familiar to the technical staff. Instrument-attached computers and imaging workstations are used by many people; thus, an active prevention policy and a good computer practice should implement the following rules:

- On Windows systems and Unix workstations, create at least two partitions within the hard disk space, leaving one for the operating system and software and another for user data, image files, etc. Block user write access to the first partition and limit user privileges to install new software or delete critical files. We would also recommend restricting Internet access so that users are discouraged to use the computers at the facility for non-experimental purposes.
- Program periodical sessions of system cleaning and hard disk defragmentation and keep the operating system updated with security fixes.
- Keep regular backups of the system and data partitions on external disks or dedicated network servers. There are several open source software options that allow scheduled and incremental backups. For each system, keep one backup copy made immediately after its original installation.
- Install security software (antivirus and firewall) that can be easily customized, requires low system resources, and does not interfere with the installed applications. Keep security threat databases regularly updated and set the program to automatically scan any device that is plugged into the computer, such as USB flash drives or external disks.

An important issue is the management of the huge amount of imaging data generated every day (Moore et al., 2008; Linkert et al., 2010; O'Donoghue et al., 2010). External disks are falling short in keeping up with the current data flow. Probably the best option is to deploy network-attached storage (NAS) servers at the facility and at the users' laboratories. Prices of computer hardware and hard disk space are falling and there are several open source alternatives available (i.e., FreeNAS, *http://freenas.org*; and Linux server, *http://www.ubuntu.com*) to set up secure, fault-tolerant (RAID), and expandable file servers. Of course, there are also commercial alternatives at a price. Such servers can provide fast (Gigabyte LAN) and transparent (NFS, Samba, FTP, etc.) access to storage space to any granted computer in the network. We emphasize that such servers should also be deployed and administered by the users' laboratories, at least by those that generate large amounts of data. Users must also understand that instrument-attached computers cannot be used to keep their imaging data or for image processing and analysis. The NAS severs at the facility should only store backup copies of the most recently generated user data in the instruments. A commonly used alternative for file transfer are USB-powered external hard disks and flash drives, which can be easily transported and connected. However, these devices may generate security issues since they are a common medium for propagating viruses, Trojans, etc.

Another issue is the annotation and archiving of imaging data for easy access in the long term. There are several image organization tools or browsers that allow quick search and view of imaging data. The OMERO project (Table 12.22.1) is an open-source initiative of the Open Microscopy Environment (OME) team designed for the visualization, management, and analysis of biological microscope images (Moore et al., 2008; Swedlow et al., 2009; Linkert et al., 2010). This client-server software allows up to 5D image archiving and

visualization organized by user, type of experiment, date, etc. Users can define tags to easily localize images and experiments and attach protocols and comments to image data for easy sharing with other users or collaborators worldwide. We consider that OMERO, at its present stage (version beta 4.2.0), is useful for advanced users to manage and share their imaging experiments. However, we think that it is still early for it to become a centralized multi-user imaging data repository administered at facilities. To get to this point, software installation and maintenance should be easier, data should transfer faster, and users and technicians should be trained appropriately.

A final issue consists in dealing with the wide variety of file formats generated by software and hardware companies to save imaging data. There are free viewers available from microscopy companies that can be used to import and export imaging data in a few formats, including tiff and jpeg. In the past, these utilities were necessary to convert data from proprietary to widely used formats, such as tiff, so that image processing and analysis could be performed. However, the experimental annotated data included in the image files (metadata) were often lost in the conversion. Bio-Formats (Table 12.22.1) is a standalone Java library that enables reading and writing most imaging data formats and is used by open-source imaging software, such as ImageJ/Fiji and OMERO (Moore et al., 2008; Swedlow et al., 2009). OME group is also promoting the use of the OME-TIFF format, one unifying and open TIFF-based image format that includes the OME-XML standard to store metadata.

## USER TRAINING

One basic purpose of a facility is, as its name suggests, helping users in harnessing the full potential of instruments that, otherwise, will require a considerable amount of individual time and effort to master. Additionally, it is desirable that regular users acquire the skills needed to operate the instruments without assistance. It is always positive to have as many skilled users as possible, since their access to instruments will not be limited by the agenda or timetable of the technical staff.

Training sessions can be organized individually or in small groups, but direct contact with the instruments is recommended in any case. Open seminars are also useful to make potential users aware of recently acquired systems, their features, and applications. Depending on the size of the audience, they could also be organized as in situ training sessions. However, in our experience, individual training sessions are always needed for a significant number of users. Until users are individually confronted with the instrument with their specific samples and doubts, efficient learning does not start. We recommend that users have a dedicated notebook and write down the protocols and procedures, so that they can be repeated easily and accurately.

The first step is usually an explanation of the basic principles of the instrument, which should be clear, concise, and illustrated with examples related to the user's experimental work. A better understanding of the instrument fundamentals helps users to get the best from the systems. Then, a quick review on security basics should be provided. Without generating alarm or excessive respect for the instrument, the user should know the hazards associated with the laser units and what to do, e.g., if an Hg pressure bulb explodes. We would advise creating a quick guide of the most common security threats and the recommended actions and placing it near the instruments. To finish this introductory session, users should know the basics of the cleaning and care of the instruments. They should understand that wiping the immersion oil from objectives, cleaning the stage, or placing back the microscope dust cover are simple routines that have a strong impact on image quality and prevent future malfunctions. They should feel as responsible for the equipment maintenance as technicians, and should know that their help is needed to maintain the instruments in good condition.

Learning software operation and instrument control can be time consuming and tedious. We recommend explaining the functions that users really need and condensing or avoiding the explanation of other features that they will probably never use. Expertise in system operation will be gradually acquired in successive sessions at rates that will vary among users. A key lesson is image acquisition. A quick review of basic concepts, such as optical resolution and zooming, dynamic range, and intensity profiling always helps. Users with limited computer knowledge would welcome basic concepts on images (2D), stacks (3D), and higher-order imaging data, color look-up tables (LUTs), lossless and lossy image compression, and metadata interpretation (*UNIT 12.21*). Further, they should be reminded of the limits imposed by scientific journals regarding image processing. Technicians should give advice and help on image

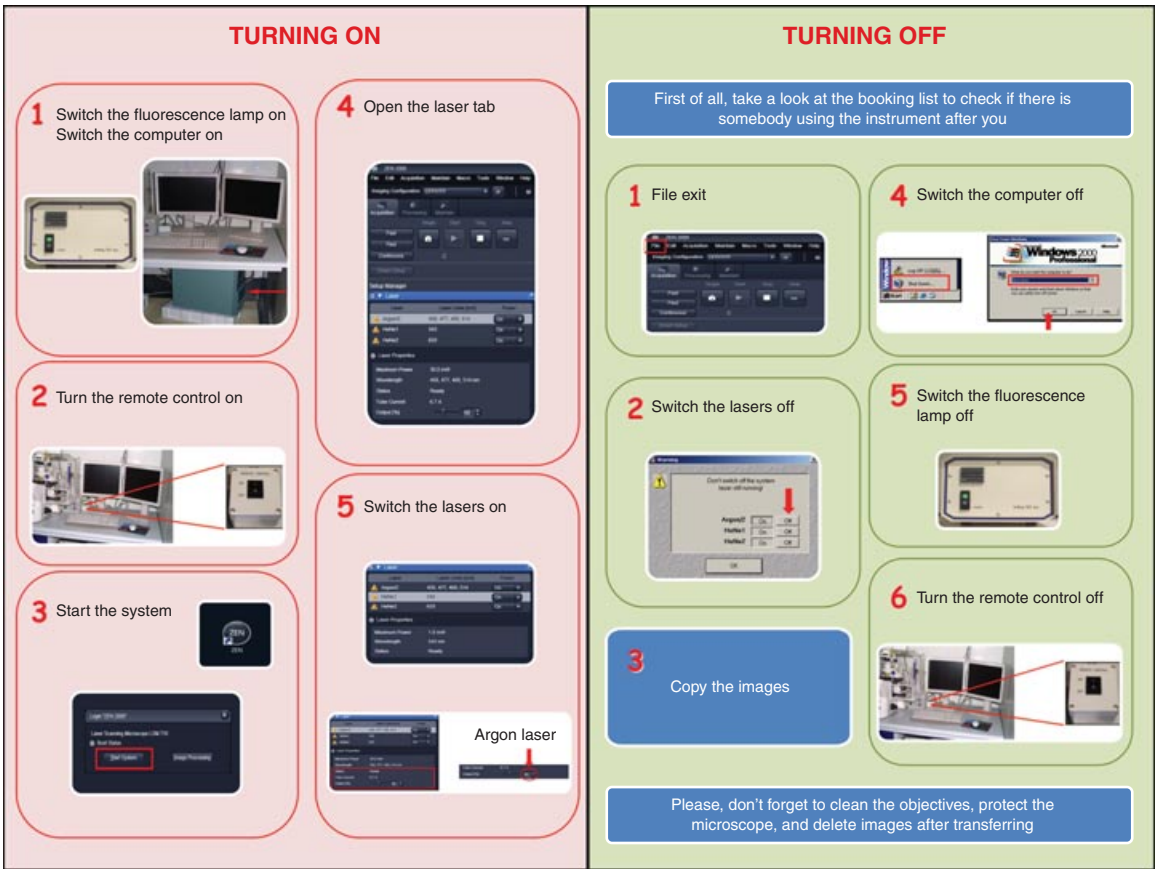


**Figure 12.22.4** Basic guidelines. Apart from quick user guides, we would recommend placing some posters close to the instruments with instructions about switching on/off, configuration, features, and basic care, which can be easily accessed in the absence of the technical staff.

processing and analysis only during the first contact of users with the software. In this sense, open seminars may be really helpful. They should be organized as practical and illustrated guides showing how to perform specific tasks, which can be later shared at the facility Web site.

If the facility allows the use of instruments out of the technician's working hours (late evenings or weekends), we advise displaying posters close to the instruments illustrating how to perform basic operations, such as switching an instrument on and off (Fig. 12.22.4). This ensures that users will not forget the procedures and will make them feel more confident.

Finally, general seminars are always a good medium to make users aware of the instrument's features and possibilities, to show the basics of image processing and analysis using real examples of daily work, to advance user knowledge on new microscopy techniques, or just to discuss the policy and future direction of the facility. Encourage users to suggest topics for seminars since that is the best way to guarantee their participation. Information on national and international microscopy and imaging courses and workshops is also welcome by users (Table 12.22.3).

## CONCLUDING REMARKS

In this unit, we have presented what we consider most important in the endeavor of setting up and running a Light Microscopy and Imaging Facility (Table 12.22.4). Microscopy and digital imaging fields are rapidly evolving and instruments are getting more and more sophisticated. Thus, facilities at research institutions are becoming necessary to facilitate the access of scientists to these technologies and also to keep the equipment working under optimal conditions. We have described a number of issues to consider for the initial setup of a facility. However, we recommend evaluating the points related to long-term maintenance and growth. A rigorous and realistic planning will greatly increase the possibilities of success. In addition to the already mentioned issues, we

**Table 12.22.4** Key Aspects of Setting Up an Advanced Light Microscopy and Imaging Facility

| Design | Equipment | Space organization | Staff | Management | Web-based management application | Computers | Training |
|---|---|---|---|---|---|---|---|
| 1. Identification of the current and future applications and instruments demanded by research groups. | 1. Define the applications and identify potential users. | 1. Avoid vibration. | 1. Practical knowledge on microscopy and imaging. | 1. Facility fee should cover the cost of the instrument, repairs (parts and labor), maintenance service contract, consumables, and technical staff salaries. | 1. To manage "online" booking, keeping detailed book, and user information. | **Maintenance** | 1. Training sessions individually or in small groups: open seminars and in situ training sessions. |
| 2. One important decision: facility aimed at instrument maintenance and user support (assistance and training) or involved in the development and setup of applications or instruments through scientific collaboration. | 2. Test and compare systems for quality, performance, robustness, and stability. | 2. Ensure the stability of the electrical installation. | 2. Background in biology, physics and/or computer science. | 2. Different charge for internal and external users, from profit or nonprofit institutions? | 2. Allowing registered users to reserve instruments anytime and anywhere. | 1. Hard disk: make partitions and regular defragmentation, cleaning and backups. | 2. Learning tasks: equipment use, basic microscopy and imaging concepts, security issues, cleaning and care of the instrument, facility rules. |
| 3. Which applications, equipment, software, and reagents should be assumed by the facility and which ones by laboratories? | 3. Value provider's technical support expertise. | 3. Optimize temperature and humidity control to avoid oscillations. | 3. Acceptable level of spoken and written English. | 3. Distinction between occasional and regular users; novice and experienced ones; or between responsible and negligent users? | 3. To notify whether assistance is needed and to report incidences. | 2. Security: block user write access in the system partition, use security software, and make regular security updates, restrict Internet access. | 3. Make some user guides and posters, which can be easily accessible. |

*continued*

**Table 12.22.4** Key Aspects of Setting Up an Advanced Light Microscopy and Imaging Facility, *continued*

| Design | Equipment | Space organization | Staff | Management | Web-based management application | Computers | Training |
|---|---|---|---|---|---|---|---|
| 4. Looking for funds to set up and keep running the facility: public or private organizations and enterprises, national or international; and facility users, from the same or other different institutes. | 4. Is it user-friendly and easy to maintain? | 4. Organize the space in dark rooms with one or two instruments, trying to achieve a comfortable and safe working environment. | 4. Organized and responsible. Work in a team. | 4. Will the facility be subsidized by the mother institution? | 4. To administer invoicing, applying the established pricing rules to each user. | **File storage and handling** | 4. A comprehensive and updated facility Web page is the best way to make the information quickly and easily available to every user everywhere. |
| | 5. Decide between versatile or specialized instruments. | 5. Do not forget about the organization of the space for some additional equipment to keep and maintain reagents, to develop cell culture, and for the office. | 5. Good at dealing with people and teaching. | 5. Some rules and limits are necessary to prolong the life-span of instruments and to make them more productive. | 5. It allows recovering instrument use statistics, which is very useful for the facility management. | 3. File storage: Network-attached storage (NAS) servers, USB-powered external hard disks, and pen drives. | |
| | 6. Evaluate advantages and disadvantages of working with systems of the same or different companies. | | 6. A scientific coordinator with knowledge and interest in the fields of microscopy and imaging is recommended. | 6. Facility planning: evaluate user and instrument statistics, make regular surveys, and also use seminars to receive some user's feedback. | | 4. Handling, annotation, and archive of imaging data and formats: Open Microscopy Environment (OME), BioFormats and ImageJ/Fiji can be good solutions. | |
| | 7. Accessories and tools: cell incubator, laminar air flow hood, fridge, freezer, software, pumps, plasticware, cell culture accessories, glass, cleaning tools, laser power meter, etc. | | 7. Keep the facility staff trained and motivated, promoting the assistance to workshops, courses, and visits to other laboratories. | | | | |

would like to stress the importance of paying attention to not only the instruments and applications available but also to the technical staff, which will support them. This is probably the most important equation to solve in the process of creating a successful and valued facility.

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