CHAPTER 14

Running and Setting Up a Confocal Microscope Core Facility

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I. Introduction

The use of core facilities for the supply and support of large shared instruments is a growing trend that will benefit all researchers, as well as those funding the projects. In these days of quickly developing technologies, no one researcher could possibly keep up to date with all the software and hardware for the applications and techniques used in his or her lab. Individual researchers don't always have time to learn all the finer points of using the new technologies, or even to research what new technologies out there are the ones they need, or that could give them the desired results. It is our service as core managers to develop and maintain facilities equipped with these fast changing technologies and to allow the researcher to concentrate on the science.

There are many facets of developing a core facility with shared equipment. In this chapter we will cover all aspects of core management, including planning the layout of

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your facility, procurement of instruments, scheduling use, training of users and operators, instrument care and maintenance, data handling, ancillary services such as protocols and sample preparation, as well as governmental issues that affect recharging. We will also cover some resources available to core managers to assist us in the job we are to do.

II. Planning Your Facility

The first step, and most likely, the impetus for starting this whole process, is the establishment of the need for confocal imaging and a user base anxious to have the technology available to them. Only when the needs are defined, is it possible to choose the microscope that will fill them. In putting together a shared facility, it is important to choose either an instrument with a lot of flexibility or one which can be augmented in the future to add all the possible capabilities you *might* need in a core. Polling the potential users, or grant applicants, for their projected needs is the most vital next step. If possible, when soliciting researchers to write the grant proposal, speak to users who will have varied applications, and cover all the possible configurations. This will help you justify the most important features for your users on the initial purchase and justify the widest variety of options.

A general idea of the requirements of your users and the basic instrument you will need is important before seeking funding, since the prices of instruments vary considerably. Funding for large equipment is available as shared instrument grants such as the National Institute of Health Shared Instrument Grant and NSF program or from individual or corporate donors. Many times matching funds from institutions are necessary in order for these grants to be funded. Strong institutional support often is the difference between a winning grant proposal and a losing one. Make sure that you understand the instrument you are requesting, and how it will support your research needs. If you sound knowledgeable about the process and the instrument, you are more apt to be funded. Before writing the grant proposal, check with your local microscope sales representatives and find a microscope you can see in action. Ask the operators, the people who actually operate the hardware and software on a daily basis, questions such as: How stable is this system? How easy is it to learn and operate this software? How long before you feel competent operating this scope? Have you needed any repairs? How was the service? Is technical assistance easy to get? Answers to this kind of question can help you rule out one or another manufacturer. Service in different parts of the country can also vary—not all technicians are created equal, so to speak—but it can be a guide.

Once you have chosen the instrument to purchase, it is vital to ensure that the environment is adequate, that you have allowed enough room to accommodate the instrument. The microscope may require a large vibration isolation table, and an access room behind the instrument for service and maintenance. You might need additional desktop workspace for the computer. Room for sample preparation nearby is helpful, but this should be far enough away from the microscope that work there will not interfere with the imaging. This allows both parts of the process to take place simultaneously.

Lasers add a complexity to the room design. The room may need extra ventilation or may need access to ceiling crawlspace or an outside vent in which to put the air-cooled laser cooling fans. This will allow you to vent the hot air out of the room, away from the lasers, the computer components, any labile or delicate samples, and you, all of which should be kept cool. If the laser is water cooled, you will need to address which water circulation or chilling system will work best. One of the three varieties circulates the water and cools it with ambient air. The second circulates water through the laser and dumps it into a drain. Another circulates the water, continually recycling it to a large chilling unit outside the building or on the roof, chilling the laser more quickly and more efficiently.

Lighting is also a concern when dealing with fluorescent techniques. It is helpful to have an incandescent light source as well as the fluorescent overhead lights so you can control them with a rheostat and keep the room dimly lit during acquisition. The lighting supplies to the confocal are also a drain on the electrical circuitry. It is necessary to consider if you have enough separate circuits that the lasers and arc lamps will not interfere with the computer and electronic components when turning them off and on. For an extra measure of safety, turn the light sources on before the computer parts and off after—do not light them while delicate electronics are on. They have been known to spike and damage your computer.

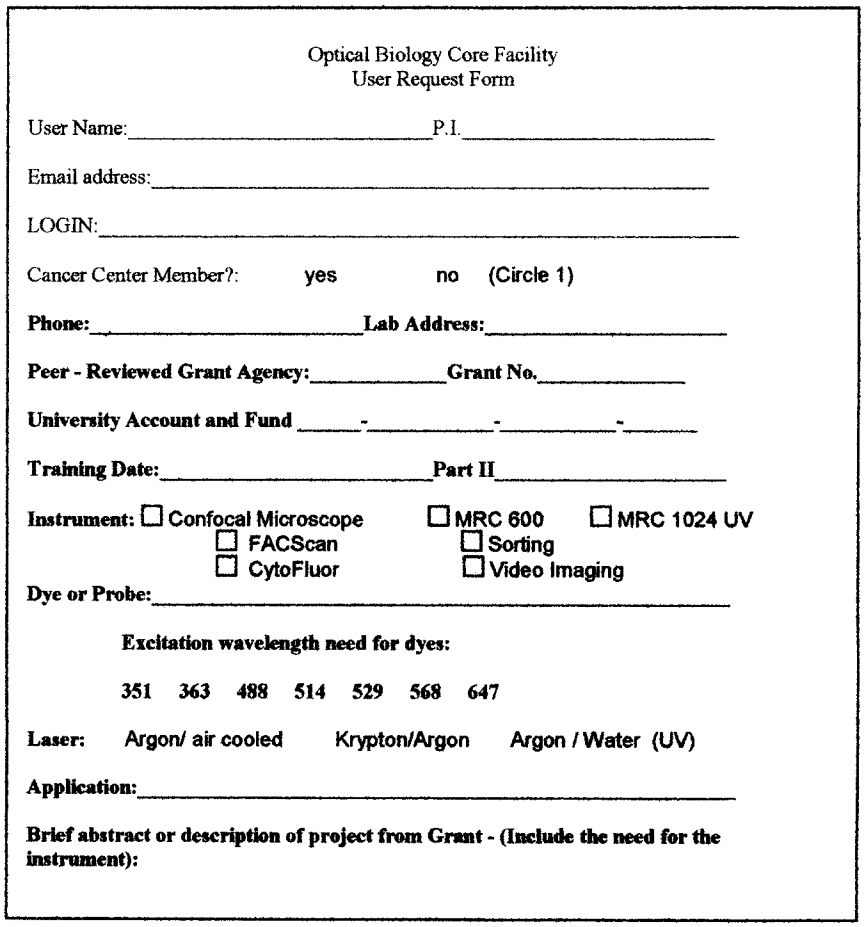
III. Scheduling

If your facility offers services to several laboratories or even different schools or colleges within your university, it is advisable to have a system of scheduling that will allow users to access it from their own computers or via the Internet. This eliminates the need for users to call *you* for an appointment or for them to walk to your laboratory door and physically write on a calendar. Programs such as Corporate Time or Windows Outlook have the capability for scheduling resources and allow users to connect to them from their computers. As general rule of thumb, the user who is properly signed up on the computer, *not the one who arrived first,* is the one with access, should there be a discrepancy. Computer calendar documentation is also a tool for confirmation of use, in case your logging system fails, or if there is question of who was using the microscope last or from which laboratory they came. Many of the newer systems include a login feature, which allows you to set up users with passwords and thus protect data and limit use by untrained users. These are important features, and it is advisable to take advantage of them when setting up your operations. An added benefit is that you have an accurate record of use and discrepancies are rare, since the computer has documented the use. Another side benefit is that the billing returns often increase when the computer takes care of the logging.

In some institutions, it is necessary to give priority to certain groups in scheduling. If this is the case, those people may have direct access to the calendar and others may have to submit an e-mail request for use. Depending on the demand for your instrument, this may cause unnecessary delays, or may be a vital step in keeping peace.

IV. Training Operators and Users

After your microscope is installed, it is necessary to become trained to operate the scope and in turn to train frequent users. All major manufacturers give you a training class or sessions with the purchase of your instrument. Parts of this training can be adapted for your frequent users, or used as an outline for the important issues to cover in your training. As a service facility manager, you may be asked to image all types of specimens for your researchers, especially if they just need one or two pictures to prove a point for a reviewer, or to document a cellular event for their own records. You will often be presented with research problems that you have never encountered before. For this reason it is beneficial to have access to a technical and applications support unit for your microscope. With the technical assistance of the manufacturer, you can face



Optical Biology Core Facility
User Request Form

User Name:_____P.I._____

Email address:_____

LOGIN:_____

Cancer Center Member?: yes no (Circle 1)

Phone:_____**Lab Address:**_____

Peer - Reviewed Grant Agency:_____**Grant No.**_____

University Account and Fund _____-_____-_____-_____

Training Date:_____**Part II**_____

Instrument: ☐ Confocal Microscope ☐ MRC 600 ☐ MRC 1024 UV
☐ FACScan ☐ Sorting
☐ CytoFluor ☐ Video Imaging

Dye or Probe:_____

Excitation wavelength need for dyes:

351 363 488 514 529 568 647

Laser: Argon/ air cooled Krypton/Argon Argon / Water (UV)

Application:_____

Brief abstract or description of project from Grant - (Include the need for the instrument):

Fig. 1 User request form for use of the optical biology core facilities.

problems you really have never seen before. Do not let inexperience scare you away. All of us at one time knew nothing about confocal imaging. Special training courses are also given at research institutes such as Cold Spring Harbor, Hopkins Marine Station, Woods Hole Marine Station, and the University of British Columbia, which holds a class in 3-D microscopy of living cells during the summer. These courses will enhance your understanding of imaging and help you offer better service.

Your frequent users will want to have access to the facilities and to take images at times when you are not available, so developing a thorough training for your users is imperative. They need to be able to accomplish their research objectives but not necessarily know everything there is to know about the microscope, so each training is tailored to the needs of that researcher. An interview with or request form filled out by the prospective user will help you establish the topics of specific interest, the research objectives of the project, and the background or experience level of the user. This type of form identifies the needs of each researcher and will help you tailor his or her training. It also helps you compile your database of users, so that communication, billing, and reporting the various uses of your facility are easier to accomplish. For an example of such a request, see Fig. 1. Before training on your system, users should be comfortable on computers or at least be able to function easily on your platform, should know the science they are working with, and should understand basic principles of fluorescence. Then you can cover these basic topics in confocal imaging:

1. The theory of confocal microscopy will give users a better idea of its capabilities and limitations. Explain to them how your system and other confocal technologies work.

2. Explain the features with which your system is equipped—such as the excitation wavelengths available on your lasers, and what dyes, stains, and probes are appropriate for those wavelengths. A table similar to that in Fig. 2, designed for an MRC 1024 UV system from BioRad, may be useful for deciding which laser and filter setup is appropriate for inexperienced microscopists. There are also Web sites available, such as one from Bio-Rad (http://fluorescence.bio-rad.com/) and one from Molecular Probes (http://www.probes.com/handbook/toc.html), that will help users understand fluorescence microscopy and determine good combinations of dyes for specific excitations as well.

3. Basic care and use of the microscope itself should be addressed, since no matter how fancy the devices attached, the images will not be good if the optics and microscope are not treated with respect and cleanliness. A more thorough discussion of instrument care follows.

4. Data collection options are a critical issue. It is important to decide where to store the images. Do you allow images to be left on your drives? How about specific methods and settings? What are your guidelines for transporting images to other computers? The most fundamental lesson is: *back it up* and have two copies of everything, whenever possible.

5. Software basic operation training should cover the options needed by that particular researcher, but also offer a taste of options the researcher might possibly want to use at

Laser	Laser Line	Default Method	Emission Filter Choices*	Dyes
Argon Ion Water Cooled	351	Blue / Green	455 / 30 405 / 35 460 LP	AMCA / FITC Alexa 350 (Em 422)
Argon Ion Water Cooled	351	Emission Ratioing	455 / 30 405 / 35 460 LP	Indo - 1
Argon Ion Water Cooled	363	Vital DNA BFP/GFP FRET	455 / 30 460 LP	Hoechst / DAPI* BFP, EBFP
Krypton Argon Mixed Gas	488	Fluorescent In Situ Triple Labeling Traditional DNA	522 / 35 540 / 30 585 / LP OG 515	FITC / PI FITC/DTAF/Bodipy/Cy3 PI / TO / AO GFP, EGFP Alexa 488 (Em519) Oregon Green (Em 524)
Krypton Argon Mixed Gas	488	Cellular Calcium Membrane Labels Neural Tracer	522 / 35 540 / 30 585 / LP OG 515	Ca^{++} Green / Fluo-3 Di A / Di O Lucifer Yellow
Krypton Argon Mixed Gas	488	Reflection	522 / 35 540 / 30 585 / LP OG 515	Reflectance
Krypton Argon Mixed Gas	488	3 Color Transmission	522 / 35 540 / 30 585 / LP OG 515	Transmission
Krypton Argon Mixed Gas	568	Cellular Calcium Membrane / Neural Tracer	605 / 32	Ca^{++} Crimson Di I
Krypton Argon Mixed Gas	568	Traditional DNA Triple Labeling	605 / 32	Ethidium Bromide / 7AAD / PI TxRed/LissRhod/TRITC Alexa 568 (Em 603) DsRed (Em 583)
Krypton Argon Mixed Gas	647	Triple Labeling	680 / 32	Cy 5, Cy 7

- Band filters - first number is the center of the band / the second the width – ie 455 / 30 means a band from 440 – 470, 15 nm above and below the center.
- LP indicates Long Pass filters
- OG – blue absorbing Long pass green filter

Fig. 2 Excitation and emission wavelengths, filters, and associated days.

some time in the future. Instrument use will increase not only when users know how to get the best out of the software, but when they discover new ways to use it.

6. Turn-on and turn-off procedures can be printed on quick reference charts near the instrument or as the home page on the Web browsers for each workstation. These guidelines are important in order to best maintain long life of your light sources and limit surge damage to computer and electronic elements.

7. One of the most difficult areas will be to instill a knowledge in your users of how to recognize emergencies, problems with the system that need immediate attention by an expert, and whom to call in such an emergency.

Of course, the bulk of your training will be on the specific software and operation of your particular instrument. Use your operators' training with the instrument manufacturer as a guide to design a training outline for your users, to cover all aspects of the operation of the confocal. However, limit user training primarily to those things that are necessary for daily operation. For example, perhaps not everyone needs to know the details of alignment on your system, especially if it is stable and needs alignment only rarely. Tailoring individual training to the needs of the researchers allows them to concentrate on getting the appropriate images they desire, and getting them quickly. However, it is also useful to present other features available on your system. This allows researchers to consider all capabilities of the system and may perhaps inspire them to expand their use to take advantage of those options in a future experiment.

V. Instrument Care

Keeping the microscope in good condition is a critical issue. No matter how fancy the electronics or computer enhancement of a system, if the optics are not cared for and kept clean, the images will not be good. Information on the importance of keeping the instrument clean, directions to where the cleaning supplies are kept, and techniques for cleaning the lenses should be included as a part of each training. Supplies are always kept within sight of the microscope. It is also a good idea to check the microscope weekly for cleanliness, and give it a thorough cleaning yourself when needed. Oil left on inverted objectives can run down into the internal parts and cloud lenses or, worse yet, corrode the objectives and ruin them. Immersion oil is very corrosive and can cause permanent damage if allowed to drain down into the microscope parts. Keep your eyes open for damage caused by users and misuse of the microscope. Don't be afraid to ask the last users for their input on the performance of the microscope when they used the instrument last. It might be possible to catch misunderstandings of microscope care, or methods of handling the equipment before permanent damage occurs. Waiting until the damage is done and the microscope is in need of costly repair limits the use of the scope for everyone and decreases the income derived from it.

The quality of images produced on your microscope can be monitored by the preparation of a slide or two containing fluorescent tapes or filter paper, and beads. Such slides are available from your microscope manufacturer or commercial suppliers, or they can be reproduced in your own laboratory. Fluorescent controls can be made from safranin

dye-impregnated filter paper, mounted with an antiquench mounting medium on a glass slide. Fluorescent beads of different colors and intensities from commercial sources can also be mounted on slides in antiquench media and used over and over for controls. Images of these controls at specified settings can be stored in a default folder, allowing you to recheck the laser intensity and the optics weekly, or when there is a question about the brightness of images. Simply reset the settings to those used in the controls and compare the images. There are also readings of voltage and power of the laser that you can read and record, to determine if the laser is losing power and in need of repair. Check with your microscope or laser manufacturer for appropriate levels and how to check them with a voltmeter.

Accurate measurements can be made with the confocal microscope by checking the calibration of each objective with a slide micrometer. Image the micrometer at different magnifications and store the information and image in a file. The confocal software package stores the calibrations, and if each image is saved with the appropriate lens chosen, you can measure structures within the images accurately. As a recheck, measure another micrometer as if it were an unknown.

VI. Data Storage, Analysis, and Handling

Storage options for images are also a huge topic of interest for core managers. Volumes of images can often be as large as 100 MB and hard-disk space on your computer can be quickly consumed by images left there. Magnetooptical drives and the advent of Jaz and Zip disks have simplified this issue, and the addition of one or more of these drives allows your users to transport images to other offline workstations for analysis. Images can even be burned onto CDs when taken to an offline system uninterrupted by other users or the network. These three options have revolutionized image storage, since they appear to be stable for many years, allow users to take their images to other workstations, and hold very large volumes of information at much less expense than previous methods. When using Zip or Jaz disks, however, it is best not to fill them to capacity, for they have been known to crash and lose all data when filled too full. Also, keep a second backup, two copies of everything important.

When multiple users have access to the system, the need to save images to individual folders or directories and to individual disks is even more critically important. Each file is important to that researcher, representing hours of work and sometimes immeasurable cost to procure. Some samples are precious and cannot be duplicated, and some require hundreds of dollars worth of sample, antibodies, and/or reagents to prepare. For these reasons, individual media are recommended for storage. Each user can keep his or her own Zip or Jaz disk in his or her possession, ensuring that all images are safe. Settings and methods can and should also be backed up onto a Zip or Jaz disk in case of system crashes. As core manager, you can decide if you want to be responsible for that level of backup, or if you want users to be responsible to back up their own images. In that case you should recommend very strongly to your users that they take that responsibility seriously.

In most instances, images can be taken from the confocal in formats that allow the users to view and change them in offline software. Most software packages available

read the common confocal formats, but if not, it is also possible to open files as raw images. Confocal Assistant, NIH Image and Scion, and Adobe PhotoShop, to mention a few, make good offline additions to your analysis capabilities. Offline analysis will allow your machine a higher level of use for imaging and increase your throughput of users. Where heavy use is a problem, you might encourage users to use these auxiliary programs on their own computers.

VII. Core Issues

Keeping track of the use of your facility is imperative with some grant funding, and for billing, but it has other benefits for everyone. It helps you know what needs your researchers have by showing what resources they are using the most. This will help you in deciding what to upgrade or update first, and what services you need to offer in the future.

It is simple enough to prepare your own forms, such as the example in Fig. 1 for record keeping, including information useful for your particular purposes, usage requests, billing, or demographics. Logs for daily use can also be used for keeping track of the microscope use by "frequent users" and of problems encountered in imaging, by including a column for comments. Quality control and maintenance on your instruments can also be monitored using personalized log sheets prepared by computer.

Confocal software systems often offer personalized features for each account, such as security logins to monitor usage, individual subdirectories to keep images safely within the user's control, and the ability for researchers to save their own methods and settings. This allows them to repeat an experiment with the same conditions, or will give them a starting place when beginning a new experiment. Information is often stored in the headers of files, indicating conditions as they were documented in the computer at the time of collection, so it is important to make sure the settings are correct when collecting the image. Six years from now, you might not remember which objective you used! Measurements taken then would be erroneous, if the correct setting was not saved originally.

Databases constructed to maintain records of all users, their usage, billing charges for each instrument for each type of uses, on and off campus, and records of all facilities help in the compilation of figures for reports and billing. With just a click you can pull up files within a time period, for just one instrument, or by one user. The time it takes to prepare the database will be well worth your while, when you need to get a statistic about usage at a moment's notice some Friday afternoon!

Charging and recharging for the use of your facility is also a primary concern. In many laboratories there is subsidy available to pay for salaries or equipment and the only goal of the recharge is to maintain the equipment and pay for supplies. Whichever scenario is the case at your institution, preparing the recharge account is not an easy task. You must justify the amount of your charge, considering all the expenses it is to cover, as well as the amount of recharge the market will bear, the standard in your part of the world, and what your clients will be able to afford. Corporations are often immune from this problem and offer the services to anyone in the company who needs them, at no charge to individual accounts. In the university setting, the situation is a little different, where the most frequent users need to bear the burden of keeping the instrument going—with

recharge money, generated by their frequent use. Along with these considerations, many granting agencies have guidelines about the amount of recharges you can assess, and whom you can charge. The National Institutes of Health (NIH) dictate that anyone using NIH granted equipment must offer it to all researchers on the campus at the same rate. The National Cancer Institute (NCI) says that anyone belonging to the cancer center supported by one of their cancer center grants is to have priority on CCC resources over other researchers, on and off campus. Researchers who contribute to the writing and support of grants for instruments often expect free use of facilities for a period of time. It is possible to meet all of these expectations using creative management, and still keep all granting agencies and your users happy. Surveys of current recharge schedules are often available through resources such as those listed in Fig. 3.

Resource	URL	Email address
International Society for Analytical Cytology (ISAC)	www.isac-net.org	ISAC@isac-net.org
Microscopy Society of America (MSA)	www.microscopy.org/	Zaluzec@Microscopy.com
Local Affiliates of Microscopy Society of America (MSA)	www.msa.microscopy.com/	Zaluzec@Microscopy.com
Purdue Cytometry Group	www.cyto.purdue.edu/	jpr@flowcyt.cyto.purdue.edu
Technical help in Microscopy and BioImaging	www.olympus-biosystems.com/technical.html	info@olympus-biosystems.com
The Bio-Rad Fluorescence Database	http://fluorescence.bio-rad.com/	microscopy@bio-rad.com
MICROSCOPY & IMAGING RESOURCES ON THE WWW	http://www.pharmacy.Arizona.EDU/centers/tox center/swehsc/exp path/m-i onw3.html	Doug-cromey@ns.arizona.edu
Recruitment Specialist for Microscopists	www.flocyte.com	flocyte@cox.net

Fig. 3 Internet resources for confocal microscopy.

VIII. Ancillary Services

Depending on your institutional needs and personal experience, you might also offer services which complement your imaging capabilities. Consultation on project design, grant proposal preparation, the writing of methods and materials, assistance with staining and specimen preparation, appropriate protocols, and reagent sources are all services needed by researchers.

IX. Resources

As your level of expertise increases, you will feel more and more comfortable in assisting your users with their project setups. Remember, as new users come to you, that you most likely have a better knowledge of your system than they do, and will be able to help them get the results they need for their research. You are the first valuable resource!

Resources such as the international organizations, listservs, and user groups are organized and available for the purposes of assisting laboratories utilizing highly technical instrumentation such as the confocal microscope. The International Society for Analytical Cytology (ISAC), The Microscopy Society of America (MSA), and its local affiliates are all available for advanced training and up-to-the-minute techniques. The microscopy listserv, the cytometry listserv, and the confocal microscopy listserv all serve as arenas for exploring new ideas and presenting questions regarding microscopy itself. The core managers' listserv deals with issues particular to the running of shared resources. All of these resources are available at no charge over the Internet and will be of immeasurable assistance when you are operating your facility. ISAC has a core managers' workgroup, organized to handle issues for core, shared resources, that is available to all ISAC members. URLs and e-mail addresses for the listservs and societies are available in Fig. 3. This is by no means an exhaustive list, but will be a starting place for your reference.

X. Summary

Confocal shared resource facilities are not only useful, but the way to go! With the equipment and its maintenance costing more and more each year, and the technology growing and expanding all the time, it is difficult, if not impossible, for individual researchers to keep pace. However, core facilities can offer the latest equipment and services for the newest techniques only when they are maintained properly and supported by researchers. Policies for the encouragement of core facilities are important considerations in the budgets of university departments. Researchers should be offered incentives to use core facilities rather than purchase their own instruments, equipment which often is neglected within just a couple of years. Core facility managers are trained and skilled operators of these highly technical pieces of equipment and are prepared to keep your facilities in the best possible condition—for the best possible results.