List of things to think about with an Imaging facility **JB Sanderson, Dec 2010**

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Main points, 1-5.

1. Vibration Isolation

– compressed air piped into room, or miniature (e.g. Clarke, Condor) compressors
- isolation of computers and monitor worktables from the microscope
- isolation of heating ducts

2. Temperature and humidity stability

– ensure rooms are small, and have sufficient tolerance ± 1ºC to give meaningful stage stability.

Methods in Enzymology (1999) 307:20-26 Part II, Practical Considerations in Acquiring Biological signals from the Confocal Microscope. Kong SY, Ko S, Lee CY, Lui PY.

Biological Signals (1996) Sept-Oct; 5(5):291-300, Practical Considerations in acquiring biological signals from the Confocal Microscope: solvent effect and temperature effect.
Lee MF, Kong SK, Fung KP, Lui CP, Lee CY.

Journal of Microscopy (2003) May; 210(2): 131-137, Reducing image distortions due to temperature-related microscope stage drift. Adler J, Pagakis, SN.

3. Local lighting.

- large, rheostat-controlled white room/cubicle light or dim red lighting; also anglepoises for local light

4. Data networking.

- Have sufficient hard disc space and dual-partition to save data first to local machine, upon acquisition, then transfer via network. Users are always cavalier with their data. Have a clearance policy!
- Buying in central file storage space backup/recovery or stand-alone; troubleshooting advice.
- You always need more electrical outlets and networking sockets than you think.

5. Activity functional work-flow diagram.

- personnel and equipment access to LMF. Traffic flow (e.g. people walking) impinging on work areas.
- distance/ease in transporting specimens from users' lab. areas to LMF
- logical workflow: (a) specimen prepn/cell-culture in LMF (b) microscopes (c) off-line image processing workstations: in LMF or separate.
- any activities to be kept separate (e.g. 'wet' and 'dry' areas; safety aspects)

A) **Building Design**
B) **Storage Requirements**
C) **Laser room factors**
D) **Lasers and other illumination sources**
E) **LMF supporting tools**
F) **Safety Issues**
G) **Networking, Computing & Image processing issues**
H) **Organisation Issues**
I) **Equipment Purchase**
J) **Education of Users**
K) **Interaction of LMF with other department/institute facilities**

A) Building design

1. The environment immediately outside the building, Locate source of stray electrical or magnetic fields or vibration (e.g. loading dock/heavy truck traffic, large electric motors or transformers, lift shafts).
2. Exact location of mechanical chases or utilities, water and drains, building elevators, stairwells, existing entrance(s) to proposed area, building loading dock, building power distribution panel(s) and electrical distribution map
3. Siting and position of LMF area – rooms in vicinity for (a) vibration/noise (b) water damage risk (c) cleanliness of air supply. Sprinkler system or local fire extinguishers (preferred).

Fit dust filters to air con units. Keep dust mat traps by doors of sensitive instruments.
Keep air con units under local LMF control, not remotely via Estates.
Position microscopes away from air conditioning vents (e.g. downdraughts from the ceiling).

Trolley-jack and lift access for import-export of equipment (e.g. large anti-vibration tables).
Corridor width; Stairs impeding movement of kit for installation and/or teaching purposes.
Statutory requirements (e.g. disabled access)

Room dimensions – sufficient to get all round the footprint of installed equipment.
Out of hours access/on-call for equipment
Door dictated by the size of equipment to be moved through the door – ? split door
Doors swinging in affecting positive/negative pressure of air con. or inducing vibration and air currents
Any light-trap (e.g. circular) entrances required?
Sliding or swinging doors – microscope bays to be individual rooms or curtained bays ?
Draught-causing potential; effect on stage movement (e.g. with ultramicrotomes in EM).
Curtains: blackout material – stray light over runner area at top; ease of flow/obstruction over floor
Window blinds or black-out capability.
Matt light paint finish (e.g. photographic darkrooms) to control lighting and prevent stray reflections, or blacked-out matt black walls for complete light exclusion.

Card access details for registered users (entry on trust, or 'lock-down' until after training approaches)
Teaching space for students (if possible). How to integrate teaching role with research role.

Floor loading capability
Floor coverings should be cleanable, impervious to oil and chemicals, non flammable and anti-static
(? Category II) integral floor and skirting.
Typical maximum floor vibrations are 3 micrometers at 5 Hz, and 6 micrometers at 10 and at 20 Hz. (Murphy).

Electrical power different phases - 1 phase and 3 phase
Three-phase power supplies required for some microscopes. Others use mains 1-phase 13 amp sockets.
Which circuits (R,B,Y) 1 phase situated on. Does load require > 1 phase circuit ? Statutory requirement: different phases should not be mixed within room.
Does any LM equipment require attention to Stray magnetic field: Horizontal and Vertical Directions, as for EMs?

Electrical interference on circuitry and physiological test-rigs. Radio Frequency interference
Regulation, conditioning or voltage stabilization of illumination sources, computers and monitors?
Electrical noise interference – avoid for CCDs and PMTs
Sufficient electrical outlets in LMF to prevent use of 4-gang extension leads.
Conduit tracking for electrical cables; Electrical wiring trays – room for expansion
Some LMFs advocate electrical services on central posts within bench islands to avoid trailing leads.
Networking cables – standard copper cable or fibre optic; cost versus speed.
Back-up power generators. Any back-up power required for sensitive instruments (as for EMs) ?

Slide preparation bench
Access to lab. fume hood
Access to cell culture hood. Where is Cell culture – nearby for transporting living samples?

Preparation area – water and power points; plumbing required (e.g. sinks, drains, safety showers, eye washes)
Lab bench area – minimum for sinks, etc. Sufficient for 'dry' and 'wet' areas.
Keep 'wet' area away from microscopes.
Area for fridge. Can normally put shelf, then small CO2-fed incubator above.
Fridge freezer space – needed for reagents that are kept by LMF for Institute: staining consistency.
Need CO2 and supply to small cell incubator – short-term culture storage.

Gas cylinders – remote or local to the room. Securing points. Storage & cylinder Access to LMF.
- if remote use warning strobes (fit an off switch over-ride)
- routing of CO2 lines to microscopes and incubators
- gas taps accessible (for short people)

Any other gas services required (e.g. filtered helium supply for drying/purging Ti-S IR lasers for 2-photon systems).
Some kit (e.g. Zeiss LSM with Pecon CO2 control) mixes the 100% CO2 in a percentage; others (e.g. 5% unmixed into Leica inverted system) does not. Have space for 100%, and 5% CO2 bottles.

Statutory safety design requirements - Hand-basin, fire-blanket, fire extinguisher cylinders.
Water supply – tap water, cooling water, deionised water.
Is Deionized and/or distilled water (location) piped into rooms, or required for specimen preparation?
Any water circulators required ? - provision for future installation and use?
Refrigeration: liquid nitrogen (also ventilation) or propane. Storage for cryo-section stubss/cells.

Location of fixed landline phones. (Also have cordless phone for work around microscopes with service and applications personnel)
Location of clocks (any stray reflections from clock face?)

Temperature Stability – exhaust of generated heat. Heat removal - Adequate ventilation to remove heat from kW output of lamp sources, computers, hotboxes in individual microscope areas. Allow 0,5 – 1kW for users.
Adequate ventilation in laser room.
Adequate ventilation and air conditioning in microscope rooms or curtained bays.

B) Storage Requirements

Storage space for spare Hg bulbs, spare objectives, (e.g) DIC accessories (? Lockable cabinets)
Small workshop area to clean objectives, change filters in filter cubes
Space for files and papers/spec sheets/operating instructions
Storage space for made-up filter blocks or box to store individual filters and spare cubes.
Fridge and/or freezer space

Wall space in microscope room/bay for individual storage space, or ? central storage facility.
Shelving behind each instrument for manuals or live-cell heating and CO2 controllers.
Tables, desks, chairs, stools
Teaching space – microscopes for basic principles to students
Photocopier/printer access. (Admin./Teaching material)
Think of future needs and expansion potential

C) Laser room factors

Central laser room or possibly separate.
Lasers – safety – to be kept in isolated room. Not common.
(e.g. Liverpool have a separate laser room because they use the LSM for luciferase.)

Siting and position of laser room - 3-5 metre limit for fibre optic cable to confocal
Dexion Laser racks in a central room with branches off – safety access only to LMF staff
Override to laser interlocks
Separate air conditioning in laser room. Estimation of heat output
Separate water feed in laser room
Sufficient electrical and network sockets. Do you need to fit dedicated 3-phase in laser room?
Dedicated laser unit and surge-protected electrical circuit.
Controlled humidity within +/- 3 degrees C of 23ºC.
Installation of computerized measurement of temperature in LMF laser room
Over-run protection if UV laser water-cooling left on - protection circuits
Laser power meter to adjust launch into fibers – does this need a dedicated second monitor in laser room?

D) Lasers and other illumination sources

Does pulsed laser for ablation (e.g. 405nm; FLIM) need to be added?
Try to avoid water-cooled lasers – condensation problems if power supply/water pressure fails
Back-up power for out-of-hours failure.
Xenon light sources for stability instead of short-arc high pressure mercury lamps
Heat extraction from lasers. (ducting in reflective foil, or wall-mounted air-con. units or air-con. unit in ceiling void with ducts above microscope bays).
Incandescent fixtures should be used to minimize possible magnetic field interference from the ballast in fluorescent fixtures. Consider effects of after-glow from fluorescent lights.
Think of access under and behind microscopes to fix lamps etc.
Overhead lights and Individual desk lights – subdued
Window blinds or black-out capability.
Track-mounted incandescent lights, controlled by dimmer controls on independent circuits
Separate lighting overhead and individual
Try to house low-light instruments in a separate room (e.g. luciferase requirements).

E) LMF supporting tools

Laser power meter to adjust launch into fibers
Multi-meter
Electronic thermometer with fine probe.

Cordless phones to enable hands-free talking to service engineers (NB mobile reception) are useful
Access to spectrophotometer to generate filter spectral curves (Brad recommends hand-held spectrophotometer with tungsten lamp for (e.g. barrier) filters with O.D. > 2)
Access to Trolley-jack and lifting equipment
Use Lascar or other USB temperature and humidity data loggers
Separate small tools - various screwdrivers (flat, phillips, and hexagonal heads), spanner, and socket sets with a good selection of small sizes, razor knife, flashlights, wrenches, wire strippers.
Hexagon spanners; Metric and Imperial Allen keys for servicing, Gas regulator spanner.

Molecular Probes/Invitrogen test samples Molecular Probes Beads (e.g.)

1. FluoCells prepared slide #2 (F14781) BPAEC cells.
2. FluoCells prepared slide #3 (F24630) mouse kidney section.
3. FluoCells prepared slide #4 (F24631) mouse intestine section.
4. FluoCells prepared slide #6 (F36925) fibroblast cells.
5. Focal check thin ring fluorescent microsperes kit 1.0um (F14791)
6. Focal check fluorescent microspheres kit 6um (F24633)

7. Tetraspeck fluorescent microspheres kit blue, green, orange 0.1um, 0.5um, 4um (T7284)
8. Spectral detection double green microspheres 6um (F36905)
9. PSF detection microspheres kit, blue, green, orange, dark red 0.1um, 0.2um, (T14792)
10. PS-speck point source kit 0.17um microsperes (P7220)

DeltaVision Calibration Kit Part No. # 52-502740-000

1. Fluorescent calibration slides
2. DeltaVision 100nm bead slide in glycerol Part No. # 52-503040-001

SGI-Pyser stage measurement micrometers - for checking/setting up digital calibration

1. England Finder – Part No. S7 02A00403
2. 20mm stage micrometer for stereo microscopes/ large FOV
3. Stage micrometer 100um (100 x 2um) Part No. S12 02A00408
4. Stage micrometer 1mm (100 x 10um) Part No. S16 02A00429
5. Stage micrometer 10mm (100 x 100um) Part No. S1 02A00400

Zeiss Service components

1. AOTF remote control Part No. 862999-0000-964
2. Laser Power Meter gigahertz Part No. 862999-0000-972
3. Test slide mirror with 0.17mm coverslip Part No. 000000-1158-109
4. Pinhole alignment mirror Part No. 453001-9062-000
5. LSM square test grid Part No. 000000-1110-203
6. LSM Test specimen scan speed Part No. 000000-1105-820

Longer 3mm Allen key – (e.g. changing camera emission filter wheel set on DV)
Head torch, hand-held torches.
Stereo microscope, or watch-makers inspection lamp, to inspect and clean objectives.
Dissecting microscope for tissue explants, zebrafish etc.
Small vacuum cleaner (e.g. Dust buster) and dust mats
Heater-stirrer
pH meter (for fixative/ immunostaining buffers if done on LMF site)
Waste pots
Fume-tight solvent bottles. Use Petroleum ether for cleaning objectives.
Booklet for Equipment to loan out
Standard transmission and fluorescence microscope to assess specimens.
Inverted phase and/or fluorescence microscope to assess cells

Cell heating equipment: Perfusion chambers (e.g. Bioptechs – DeltaT dish; Intracel, Warner, Dvorak Stotler,
Objective heaters (Bioptechs, 20-20 Tech, Bioscience tools)
Objective water-cooling jacket - Okolabs
Pecon Bachhoffer chambers, Petri dish stage warmers (e.g. Instec)
CO2 monitor for stage coverplate (e.g. see http://www.cellmc.com/ref/ctcsOct07.htm)
Galvo Z-stage, Piezo objectives, Dipping Objectives

F) Safety Issues

Storage for flammable cleaning materials (e.g. petroleum ether, ethanol).
Storage for COSHH substances – stains, fluorophores.
Hand-basin, fire-blanket, fire extinguisher cylinders. Separate lab. sink area.
Biological containment and disposal of sharps, glass slides etc.
Separate disposal containers (large 1 litre beakers/small swing bins) for lens tissues
Eyewash and first-aid safety kits
Ergonomics – chairs with sufficient reach to all microscope heights for the smaller user.
Dedicated first aiders
Safety lights (e.g. ‘laser on’) and safety signs required.
Sighting of fire alarms (e.g. for hard of hearing).

G) Networking, computing & Image processing issues

Enough separate circuits for lasers, microscopes, lighting, arc lamps, and computer power
Enough Off-line computers for software.
Computers – sufficient sockets and internet/network sockets
Streamline on software options: minimize - fewer learning curves. Intuitive – easy use, Cross platform
Commercial versus Non-commercial. (e.g. see Scott Fraser paper).
Close links and support from computer and IT services?
Particular problems integrating Linux-based DV systems into PC-based networked data-servers
IT data handling, audio-visual and 3-D packages.
Databases for large datasets (eg OME; nBIRN; Karten, HJ paper)

H) Organisation Issues

Booking data base - to also bill and manage cost and finance (eg FaMS 1,3 – Scionics)
(Set up custom database and lmf-user mailing list to manage/monitor instrument use
Database access either on trust and open-access vs. 'lock-down' system regards use and training)

File server space – stand-alone server with modular add-on capability for LMF, or buy in server space in central university computer services server space. What policy for short-notice upgrade/space increase?
LMF list server – mailing list for internal communication with users (e.g. lmf@mpi-cbg.de; lmf@shef.ac.uk)
lmf-public fileserver space (e.g. \\file-srv2.mpi-cbg.de\lmf-public). For users to log on to collect teaching material for PhD student, or similar staff, microscopy training course.
Confocal, Microscopy, ImageJ Listserver (Share, glean information from these list-servers)

Maintain records, ordering/purchase – e.g. Salary survey discussion on listserv.
Service records – equipment change
Recharge models – research groups, university, government input/funding
Advertise your services – webpage, Devolve teaching role - webpage

I) Equipment Purchase

Service contracts – 4/5 year full service warranties built into purchase price.
Recharge systems - Cost matrix budget amounts
Policy on depreciation and/or inflation calculation.
Full economic costs, or partial cost recovery.
Updates, upgrades and gradual replacement of hardware of all imaging systems
Updates, upgrades and gradual replacement of all computers
Updates, upgrades of current imaging and image processing software
Repair and replacement of broken or damaged objectives

J) Education of Users

Good practice advisory service.
Safety aspects for Good Lab. Practice. Lamp and laser safety; ergonomics; avoiding eye-strain.
Practical teaching courses - principles of microscopy, instrument operation.
Intra- and Internet Web material
PC laptop and LCD projector for teaching preparation
? separate room for training (with lectures/microscopes) independent of research activity?

Availability of standard brightfield/phase/DIC/fluorescence microscopes
In-house teaching (eg lmf-public fileserver space)
External courses – worldwide, Pawley, FEBS, RMS etc.
Internet resources – e.g. Mention microscopy fsu-primer
Manufacturers internet information – Chroma; Nikon, Olympus, Zeiss Cellscience database etc.

K) Interaction of LMF with other department/institute facilities

Lockable room for Beta-testing new equipment – commercial/industrial development policy
Optical Technology – development and evaluation of new equipment (separate or within LMF)
Electronics and mechanical workshops
Computer services - software support, networking, internet access, data storage/retrieval
- image processing, analysis, deconvolution
- high throughput screening

Administration - finance/book keeping, purchasing, secretarial support
Photolab – printing, video editing, posters, teaching materials
Library – literature, teaching resources
Central glass washing, Media kitchen/solutions