Confocal Microscopes – Laser Safety Considerations

1. What they are?

Confocal microscopy is an optical imaging technique used to increase optical resolution and contrast of a micrograph by adding a spatial pinhole placed at the confocal plane of the lens to eliminate out-of-focus light. It enables the reconstruction of three-dimensional structures from the obtained images. This technique is used in a number of Schools at the University both in Science and Medicine Faculties.

The following types of confocal microscopes are commercially available:

- **Confocal laser scanning microscopes** use multiple mirrors to scan the laser across the sample and "descan" the image across a fixed pinhole and detector.
- **Spinning-disk confocal microscopes** use a series of moving pinholes on a disc to scan spot of light.
- **Microlens enhanced or dual spinning disk confocal microscopes** work under the same principles as spinning-disk confocal microscopes except a second spinning disk containing micro-lenses is placed before the spinning disk containing the pinholes. Every pinhole has an associated micro-lens. The micro-lenses act to capture a broad band of light and focus it into each pinhole significantly increasing the amount of light directed into each pinhole and reducing the amount of light blocked by the spinning disk.
- **Programmable array microscopes (PAM)** use an electronically controlled spatial light (SLM) that produces a set of moving pinholes. The SLM is a device containing an array of pixels with some property of the individual pixels that can be adjusted electronically.

The University has several of the first two types.

2. Laser Safety – What hazard do confocal microscopes pose?

In terms of laser safety, most confocals are low risk* because, although the laser beam is accessible between the objective and the sample stage, the distance between the two is usually very short and crucially, the laser beam is highly divergent and therefore the power at this point is likely to be below the Class 3B level. Generally there are no other points at which the laser beam is accessible.

Schools should assess the situation for each confocal microscope and take into consideration information from the manufacturer and the worst case scenario of the most powerful laser at the various wavelengths. It may be appropriate to measure the power output at the objective to demonstrate that it is below the Class 3B minimum. If the section does not possess a power meter, the Safety Office can offer contacts in other Schools that do have such equipment.

If the power level at the objective is measured and found to be within the Class 3B or 4 range, the MPE must be calculated to decide whether this poses a risk to users and others and therefore the standard safety precautions for a designated laser area must be put in place. Refer to University Laser Safety Code of Practice and the next section.
Summary of hazard:

*Low risk Confocal – accessible laser power below the Class 3B minimum

High risk Confocal – accessible laser power within the Class 3B or 4 range.

3. What School arrangements should be in place for confocal microscope safety?

As for all work equipment, the responsible person for the confocal microscope must ensure that a risk assessment and safe operating procedures are in place. These must take account of all operations – use, cleaning, maintenance and servicing.

For equipment in a Designated Laser Area by nature of the risk it poses in normal operation (i.e. high risk equipment), the School Laser Supervisor should carry out an annual laser survey to check the appropriate control measures are in place.

For equipment that is not in a Designated Laser Area because in normal use it does not pose any risk (i.e. low risk equipment), there should be a procedure to cover instances where the laser compartment has to be accessed for maintenance or re-alignment. Only authorised persons (generally restricted to service engineers unless university members have been appropriately trained and authorised) should be permitted to work with the laser compartment open. Unauthorised access should be prevented during this time – for example No Entry signs on the door, knock and wait if entry required, adequate screening of any transparent door panels.

4. Training and authorisation of users

If in normal use, the confocal is in the low risk category (accessible power below Class 3B), it is not necessary for users to complete laser safety training as there should be no circumstance when they are exposed to a hazardous laser beam. However, operational training will be necessary anyway and users should be made aware that there are high class lasers contained within the equipment and that they must not under any circumstance attempt to access the laser compartment or be present whilst the laser compartment is open.

If in normal use, the assessment of risk has shown that the equipment is high risk (accessible power in the Class 3B or 4 range), the user should be registered with the School Laser Supervisor, using the standard University user registration form and should be trained in laser safety, both the general principles (on-line course) and the specific precautions in relation to the actual equipment they will be using. There should be a record of training and achieving competency for each user, in line with the School system.

5. University policy regarding registration and monitoring of confocal microscopes

Registration

The University Safety Office maintains an inventory of all Class 3B and 4 lasers across the University.
The usual laser registration form should be sent to the Safety Office by the School Laser Supervisor for any confocal microscopes brought into use in their School. Accompanying paperwork (risk assessment and laser survey form) should allow Schools to confirm the power level in normal use at the objective and whether any operation or modification of the equipment permits potentially harmful exposure. If so, the risk assessment and laser survey form must indicate the control measures in place to minimise the risk, both to users and others who may enter the room.

6. Monitoring of Confocal Laser Safety

The University Safety Office, in conjunction with the University Laser Safety Adviser, will monitor the safety arrangements in place for confocal microscopes where the risk of exposure to a harmful laser beam exists in normal use. The same strategy will apply as for other open-beam work with the higher classes of laser. Usually, this will form part of the annual laser inspection for the School but may be a specific visit if significant changes or modifications are made.