**Task/ Work:** Leica SP8 Tandem Head inverted confocal system

**Location (Room/ Building):** Building X, Room B01

**Date :** X/Y/ZZZZ

**Reference / Version :** V1

**Review Date :** Annually, or at change of use

**University Laser Safety Officer :** Dr Ian Haslam, ian.haslam@manchester.ac.uk

**Local Laser Safety Advisor :** laser safety advisor’s name

**Issued under the authority of :** PI/Lab manager’s name

**Purpose & Scope**

This document describes safe working procedures, the use of controls identified in the risk assessment, and plans for any reasonably foreseeable incidents. This document covers the use of the Leica SP8 Tandem Head Inverted confocal system located in lab B01 of Building X. This system employs class 3B lasers and RG3 LEDs. These rules implement the University’s laser safety policy at a practical level and form part of the University’s duties under Section 2(3) of the Health and Safety at Work etc Act 1974.

**Introduction & Description of Lasers**

Laser light is focussed onto the sample via the objective and is raster scanned over the sample using a galvanometer in the scan head in order to excite fluorescence from the sample in a z-section. The stage is moved up and down to refocus the laser to different z-planes within the sample. Fluorescent light emitted from the sample is collected by the objective and passes towards the detection system via a pinhole that is aligned confocally with the focal plane of the laser. Non-descanned laser light that passes through the sample is also collected and guided onto a detector.

The SP8 upright confocal system employs the following Class 3B lasers:

(1) Blue Diode laser, 405nm, CW, 50mW (<6mW at stage)

(2) White light laser, 400nm to 700nm, <500mW

(3) Micropoint UV ablation laser, N-pumped Dye laser, Pulsed, 365-656nm, 15Hz/50uJ

The SP8 Inverted system also employs blue, green and red RG3 LEDs in a CoolLed pE-300 illumination system.

Confocal lasers are housed in enclosed boxes under the microscope and are completely enclosed in interlocked enclosures, fibre optics and flight tubes before delivery to the scanhead. The Micropoint UV ablation (laser 3) is housed separately and is delivered directly to the rear port of the microscope via a fibre optic cable and a dedicated optics module. The scanhead is mounted onto the back of a Leica DMi8 inverted microscope and allows laser light through to the sample via objective lenses mounted on a turret. A condenser module and circular beam shield above the stage blocks laser light that passes through the sample.

**Justification for Open Beam Work**

The Class 3B laser path is open between the objective and a condenser, however the gap between the objective lens and the condenser is less than 10 cm making direct viewing of the beam physically impossible. A circular beam shield attached to the condenser reduces the risk of short angle specular reflections reaching the users eyes. An environmental control chamber placed around the microscope maintains a safe working distance of at least 20 cm from the laser at the objective. Microscope is configured so that laser light cannot enter eyepieces.

**Authorised Users / Responsibilities**

Only persons who are adequately trained (by attending an SP8 inverted confocal training Session run by a member of Bioimaging staff and being familiar with these local rules) and authorised as listed in the appendix to these local rules may work with the enclosed laser beam. Users must undergo a period of supervised use until both the user and the supervisor are confident that the user is competent to use the system unsupervised. Users must sign the H&S declaration sheet for this microscope to confirm that they have received training and read these local rules.

Users are only required to take a Laser Safety Awareness Course (THS42e) and do not need to undergo laser worker medical screening or complete a laser worker induction checklist as this system is a Class 3B system ‘with exemptions’.

Only Bioimaging staff (X, Y, and Z) or a qualified service engineer may perform basic maintenance on the system (as described in the user manual). Bioimaging staff have all taken the laser safety training course (THS42e).

Only qualified service engineers are permitted to perform laser replacement / alignment.

**Laser Controlled Area**

Room B01 is a laser lab measuring approx. 2.5m x 2.5m, with walls that are painted matt white and no windows. This lab is used exclusively for the SP8 inverted confocal system. There is a user operated ‘Room in Use’ luminaire above the door outside the lab.

Beams are fully enclosed (in a class 1 enclosure) except between objective and condenser. However it is physically impossible to get eyes in line of the beam. The system is configured so that laser light cannot be directed through the eyepiece. These engineering controls mean that exposure to the beams is unlikely.

**Protection Measures / PPE**

There should be no accessible radiation above ELV/MPE. The Class 3B laser path is open between the objective and a condenser, however the gap between the objective lens and the condenser is less than 10 cm making direct viewing of the beam physically impossible. A circular beam shield attached to the condenser reduces the risk of short angle specular reflections reaching the users eyes. An environmental control chamber placed around the microscope maintains a safe working distance of at least 20 cm from the laser at the objective. Microscope is configured so that laser light cannot enter eyepieces.

No PPE required by general users.

Laser alignment will only be performed by a qualified service engineer, who will provide his own appropriate PPE. No one else will be present in the room during alignment.

**Procedures**

Five types of procedure will be executed during usage of the SP8 inverted confocal system:

1. Confocal microscopy or laser ablation of non-hazardous samples.

2. Sample viewing using the pE-300 CoolLed LED system.

3. General use of the lab and the SP8 system.

4. Confocal imaging or laser ablation of live samples (Biohazard ClassI/II samples).

5. Basic Modification, Maintenance or Reconfiguration of the SP8 system.

6. Replacement / Alignment of the SP8 lasers.

**1. During SP8 confocal/Micropoint Laser operation**

Before activating the laser

(i) Remove all jewellery

(ii) Ensure that the following components are securely in place:

- All objective lenses (or port caps to cover open ports)

- Beam splitter turret

- All parts of the laser enclosures

- Fibre optic cables connecting the laser to the microscope.

- The condenser

- The temperature control chamber

While the laser is active

Do not stare at the sample or put your face near the sample stage.

Do not introduce fingers or other appendages into the beam path.

Do not remove microscope objectives or port covers.

Do not disassemble any part of the SP8 system (including the beam splitter turret, laser enclosures, condenser, temp chamber).

Do not place reflective or magnifying objects into the beam path

Do not attempt to adjust the sample manually.

Do not attempt to change samples.

**2. During operation of the pE-300 LED system**

Do not remove the fibre optic cables from the LED box or the microscope.

Do not stare at the LED light at the sample stage.

Do not attempt to change or adjust the sample manually when the LEDs are on.

**3. General Rules for normal use of the lab and SP8 system**

• Users must submit risk assessments, GM/Biohazard risk assessments, local rules and COSSH forms for their experiments before they are allowed to use the SP8 system. Any specific rules for a particular experiment (as set out in the individual risk assessments) must be followed.

• Clear up any spillages or breakages immediately and report them to a member of XXX staff.

• Only Leica immersion oil should be used with the oil objectives on the SP8 system (but the special oil provided can be used when doing experiments at 37°C).

• Use lens tissue to remove oil from objectives after use.

• Avoid contaminating the area with biological or chemical substances from samples.

• Take at least one 20 minute break every three hours to avoid eye strain.

• In normal circumstances the room is designed for a maximum of 4 people.

• Do not eat or drink in this microscope laboratory.

• LAB COATS MUST BE WORN IN THIS ROOM AT ALL TIMES.

**4. When handling live samples**

• If your sample requires a BioCOSHH form then a specific section must be written to cover the interaction of you sample with this microscope, transport of the sample to the microscope lab and disposal of the sample afterwards.

• Live samples must be transported to and from the facility in a suitable sealed container. Petri dishes and well plates containing live samples should be sealed using parafilm.

• Protective eye-wear, clothing and gloves must be worn at all times if

this is required by the risk assessment for the sample.

• Gloves must not be worn when opening doors and curtains or typing on keyboards (if necessary a gloved hand can be used to handle the sample and a non-gloved hand used for these other tasks).

• slides or other consumables used in the experiment must be soaked in detergent (e.g. Distel) for 24hrs before being autoclaved and disposed of (where possible).

• It is the user’s responsibility to remove biological waste from the facility and dispose of it appropriately

• All work surfaces and equipment that come into contact with the cells must be washed with detergent (where possible).

**5. Basic Modification, maintenance and reconfiguration of the SP8 system must only be carried out by Bioimaging staff or a qualified service engineer**.

• Before disassembling any part of the SP8 system the laser power must be turned off and the power keys removed.

• Before laser power is restored all fibre optics components, the polychroic beam splitter turret, condenser and laser enclosures must be replaced.

**6. Laser replacement / alignment must only be carried out by a qualified service engineer.**

• When aligning the laser, before activating the laser

(i) inform all users of the lab that laser alignment is taking place (via the booking system)

(ii) Remove all jewellery.

(iii) Switch on the external laser warning light.

(iv) Additional warning signage reading ‘Laser alignment in progress, Do not enter’ must be placed on barrier tape across the lab door.

(v) Appropriate laser safety goggles for the laser to be aligned must be worn (These are the responsibility of the service engineer).

(vi) The laser must be set to the lowest power practicable.

• The service engineer must follow his own safe operating procedures for laser alignment, must complete a laser permit to work and must sign off the system as safe to use once work is complete.

• Before taking off laser safety goggles and switching off the lab warning light ensure that

(i) The laser is turned off at the key switch

(ii) All SP8 components have been securely reassembled.

**Summary of Hazards**

Class 3B laser beam hazards

RG3 Radiation from LED light sources.

Group I/II biological agent hazards from some samples

Possible GM/Chemical/Radiochemical hazards from some samples

**Contingency Plan**

**Exposure to Laser / LED light**

In the event of an accident involving eye exposure to a Class 3B laser beam or RG3 LED light do the following:

(1) Turn off the power to the laser at the mains.

(2) Inform the LLSA (XXXX)

(3) Take the person to the MRI Casualty, Oxford Road Manchester along with a completed emergency laser information pro forma (for the SP8 Inverted confocal) as soon as possible and within 24hrs of the incident.

(4) Leave all equipment as it was when the accident happened.

• In nearly all cases where eye exposure to a class 2 or class 3 laser (or RG2 or 3 LED) has occurred, there is no permanent damage to the eye. The person exposed may experience pain, strong after images and blurred sight. These symptoms tend to disappear with time.

• It is important that the person does not rub their eye after exposure as this can lead to corneal abrasions.

• Those exposed to the laser beam / LED light should not drive to hospital themselves but get a colleague or a taxi to take them.

• If you require assistance you should

(1) Use an internal phone to call security on Ext. 69966 (at any time).

(2) State the location and nature of the incident/accident

(3) Request an ambulance to take the casualty to Manchester Royal Infirmary

(4) Take the completed emergency laser information sheet with you.

**Spillage of a group 2 biological agent**

In case of a spill, detergent should be poured on the spilled reagent and it should be mopped up with a paper towel and disposed of appropriately. For a major spill deploy the biological spills kit and inform XXXXX

**Scheme of Work Approved by:**

Name: ………………………… Signature:………………………… Date:…………

**THIS DOCUMENT SHOULD BE REVIEWED ANNUALLY**

**User Declaration**

*I have read and understood this document and agree to abide by its requirements at all times.*

*I understand that in the event of any malfunction, or suspected malfunction, of any part of the laser system or its security and safety systems that the experiment must be stopped immediately, the laser switched off and the matter reported to local staff supporting the experiment. I accept that we are all jointly responsible for one another’s safety and undertake not to knowingly permit the infringement of these Rules and Procedures by others.*

**Authorised Users**

**Name Signature PI Signature Date**

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In case of emergency call your nearest first aider, outside of normal working hours call Security on 0161 306 9966.

If an ambulance is needed, telephone 999 stating clearly the full postal address (XXX), and what is wrong with the casualty, together with your name. Also inform building reception (XXXX) and arrange for somebody to meet and direct the ambulance staff to the casualty.

In the event of an accident involving eye exposure, as soon as possible and within 24 hours of the incident, take this completed card and any relevant risk assessments to:

**Manchester Royal Eye Hospital, Oxford Road, Manchester, M13 9WL**

Emergency Eye Department opening times: 08:00-20:00, 7 days a week

Outside these hours call: 0161 701 0249, or attend general A&E at Manchester Royal Infirmary (address as above)

Do not drive yourself. Get a friend or colleague to take you, or use a taxi.

It is important that the affected person does not rub their eye after exposure as this can lead to corneal abrasions.

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| Laboratory Address: |  |
| Laser classification: |  |
| Type: Pulsed, Continuous Wave |  |
| Wavelength (nm) |  |
| Effects on Eyes of excessive exposure. | Delete as appropriate:  180 – 315 nm: Photokeratitis  315 – 400 nm: Photochemical cataract  400 – 780 nm: Photochemical and thermal retinal injury  780 – 1400 nm: Cataract, retinal burn  1400 – 3000 nm: Aqueous flare, cataract, corneal burn  3000nm – 1 mm: Corneal burn |
| Pulse energy (duration, peak power, repetition frequency) |  |
| Circumstances of accident / injury: |  |
| Time & Date of Injury |  |
| Eye affected: Left / Right / Both | |
| Were protective goggles being worn? Yes / No | |