

## Standard Operating Procedure

**SOP022**

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Title: USE AND MAINTENANCE OF THE OLYMPUS CKX41 INVERTED MICROSCOPE

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Location: CBE Laboratories

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### **1. PURPOSE**

To describe the safe use and maintenance of the CKX41 microscope

### **2. SCOPE**

This SOP applies to the operation and maintenance of the CKX41 Microscope in CBE laboratories (T208b) for (1) transmitted light observations (phase contrast, bright field) and (2) blue, green/ultraviolet excited fluorescence observations of specimens. The procedure describes the process for switching between observations using a slider to image the cells cultured on glass or tissue culture treated plastic, cell suspension or cell aggregates and/or examine colour-stained or fluorescence cellular specimens. This SOP also describes procedures for capturing the images of the inspected specimens using an attached microscope digital camera system

### **3. REFERENCES**

1. Operator Instructions CKX41/CKX31 culture microscopes
2. Operator Instructions: Reflected fluorescence system for CKX41
3. Operator Instructions U-RFLT50 power supply unit
4. Operator Instructions: DP12 Microscope digital camera system
5. SOP038; Biological Spill Responses
6. SOP003; Disposal of Biological healthcare Waste

### **4. SPECIAL NOTES – HEALTH & SAFETY**

**NOTE:** Improper use of the microscope could result in personal injury to the user and/or damage the equipment. In particular, the microscope can be seriously damaged by improper care of lenses. This SOP must be read in conjunction with the operator instruction manuals in order to avoid damaging the microscope lenses.

- (i) The microscope stage must be cleaned after use. Wipe the contact surface of the stage with a paper towel soaked with 70% IMS.
- (ii) Avoid spilling culture liquid or water onto the stage, objective or microscope frame. If liquid is spilled, set the main switch to off and disconnect the power cord from the wall outlet. Remove the spill by wiping with a paper towel soaked with 70% IMS.
- (iii) Ensure that the equipment connection cables do not come in to contact with the lamp housing. The surface of the lamp housing of the illumination column and fluorescence system will become extremely hot during operation.
- (iv) Ensure that the microscope is covered with the designated dust cover when not in use.

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**CAUTION:** Switch off both the main switch and the power supply before covering the microscope. Ensure that the lamp housings for both microscope and fluorescence system are sufficiently cool before covering

- (v) Avoid potential shock hazards and burns when replacing the light bulb or burner. Before replacing, set the main switch to off then disconnect the power cord from the wall outlet.

**CAUTION:** If the bulb or burner needs to be replaced during the operation of the microscope or immediately after its use, allow the lamp housing to cool before touching.

- (vi) Ensure that the reflected fluorescence system is placed on a flat surface so that ventilation opening on the bottom panel is not blocked.

**CAUTION:** Do not install the system on a 'soft' surface.

- (vii) Ensure that there is ample free space around the lamp housing (especially above and below). The lamp housing parts get extremely hot during operation.

- (viii) Avoid viewing the excitation light during observation. Although the lamp housing incorporates a UV cut filter the excitation light is not hazardous.

- (ix) Always wear appropriate PPE ie lab coat and gloves for handling specimens for observation with the microscope.

## **5. RESPONSIBILITIES**

- (i) All personnel that use the Microscope are responsible for the proper use and maintenance of the instrument as outlined in this document. Users must ensure that the working area is kept clean during work and disinfected after the work has been completed (see section 4).
- (ii) The Laboratory Manager/ responsible person are responsible for the scheduling of service and preventative maintenance with authorized service representatives. External maintenance and servicing of the Microscope can only be performed after it has been suitably disinfected (see SOP 003) and a 'Decontamination Certificate' has been issued (a proforma is available on the CBE website).
- (iii) Responsible Person/Lab Manager has a duty to provide users of the Microscope with the following:
- Information on the hazards and risks to health
  - Instruction in safe procedures
  - Training, where necessary; and effective supervision to ensure, so far as is reasonably practicable, that Microscope is operated without risks to the health of employees and other persons, i.e. including students and visiting research workers.

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### **6. EQUIPMENT AND MATERIALS**

- OLYMPUS CKX41 INVERTED MICROSCOPE with 4x, 10x, 20x & 40x lens and phase contrast slider (pre-centred)
- CKX-RFA: Fluorescence illuminator: Provides fluorescence observation (Ultraviolet, B and G excitation) for CKX41.
- DIGITAL CAMERA SYSTEM FOR CKX41& ALL ADAPTERS

### **7. PROCEDURE**

#### **7.1 Operating Procedures for using the Microscope:**

**NOTE:** Refer to Annex 1 for schematic of the microscope. Refer to Operator Manual for more detailed instructions of the procedure

- (i) Set the main switch to “ON” and turn the light intensity control knob to obtain appropriate brightness.
- (ii) Place a specimen on the stage.
- (iii) Turn the revolving nose-piece to bring the 4X objective into the light path. **NOTE:** Ensure that the revolving nose-piece is turned until it clicks.
- (iv) Adjust the interpupillary distance of the eyepieces.
- (v) Adjust the dioptre of the eyepieces.
- (vi) Bring the required objective into the light path and focus on the specimen. **NOTE:** Always localise your sample with 4X objectives before further observation using high magnification.
  - For phase contrast observation with 4X objectives, use the left position of the phase slider
  - For phase contrast observation with 10X, 20X & 40X objectives, use the centre position of the phase slider

**NOTE:** For observation of a non-dyed with brightfield, stop down the aperture iris diaphragm. For phase contrast observation, set the aperture wide open.

#### **7.2 Operating Procedures for Using the Reflected Fluorescence System**

**NOTE:** Refer to Annex 2 for schematic of the fluorescence microscope layout. Refer to Operator Manual for more detailed instructions of the procedure.

- (i) Use the transmitted observation to search for the observation target position on the

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specimen.

- (ii) After locating the observation target position, turn off the transmitted light illumination.
- (iii) Set the main switch of power supply unit to "ON". Wait 5 to 10 minutes to allow the system to stabilise.
- (iv) Engage the fluorescent mirror matching the specimen in the light path.
- (v) Open the fluorescent shutter by engaging the filter slider to the middle position.

**NOTE:** To adjust the observation brightness, engage the filter into the light path by sliding the slider to the left position.

**NOTE:** When not performing an observation, set the filter to the shutter position (right position) to prevent the specimen fluorescence colour fading.

### 7.3. Procedures for using the DP12 Microscope Digital Camera System

**NOTE:** Refer to Annex 3 for schematic of the digital camera layout. Refer to Operator Manual for more detailed instructions of the procedure.

**NOTE:** Ensure camera is OFF when inserting and removing SmartMedia card

- (i) Insert a Smart Media card into the control box.
- (ii) Press the main switch button on the control box.
- (iii) Press the MODE button so that the arrow on the screen points to "REC AUTO".
- (iv) Ensure that the specimen is in focus and then press the EXOPSE button to record the picture.
- (v) Images may be retained in laboratory notebooks.

### 7.4 Maintenance Procedure

#### 7.4.1 General Maintenance

- (i) If the lens becomes contaminated with any substance, contact the laboratory manager for instructions on required cleaning procedures.
- (ii) Clean all glass components by wiping gently with gauze. To remove fingerprints or oil smudges, wipe with gauze moistened with 70% IMS.

**CAUTION:** Do not use organic solvents to clean microscope components other than the glass components (see below).

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- (iii) Clean non-glass microscope components using a lint-free, soft cloth moistened with a diluted neutral detergent.
- (iii) Do not disassemble any part of the microscope - this could result in malfunction or reduced performance.  
  
**CAUTION:** Do not disassemble or dismount any part of the system without consulting the Laboratory Manager/Responsible Person.
- (v) When not using the microscope, reduce the brightness, switch off both the main switch and the power supply, and keep it covered with a dust cover.
- (vi) To minimise the interferences of blowing bulb to the microscope usage, two spare bulbs were kept in the lab. Fill in the maintenance record (Section 8) and inform the lab manager if the spare bulb was used.
- (vii) The mercury burner has an average service life of 100 hours. The hour counter for the used life time is displayed on the power supply unit of fluorescence. When the hour counter on the power supply unit indicates 100hrs, replace the burner with a new one (for replacement instructions refer to Operator Instructions for Reflected Fluorescence System).

### 7.4.2 Microscope Malfunction

- (i) The Laboratory Manager should be informed if any part of the equipment fails or malfunctions. With permission of the Lab Manager the user should consult the Operator Instruction Manuals for fault finding and troubleshooting procedures.
- (ii) All problems and corrective actions should be recorded in the maintenance log (Section 8).
- (iii) If the equipment fails to work or malfunctions and cannot be rectified according to troubleshooting procedures detailed in the Operator and Users Manuals the following should be observed:
  - Inform the Lab Manager.
  - Attach a 'Do Not Use' notice.
  - Contact the manufacturer for advice. External maintenance and servicing of the equipment can only be performed after it has been suitably disinfected (see SOP003) and a 'Decontamination Certificate' has been issued (a proforma is available on the CBE website).

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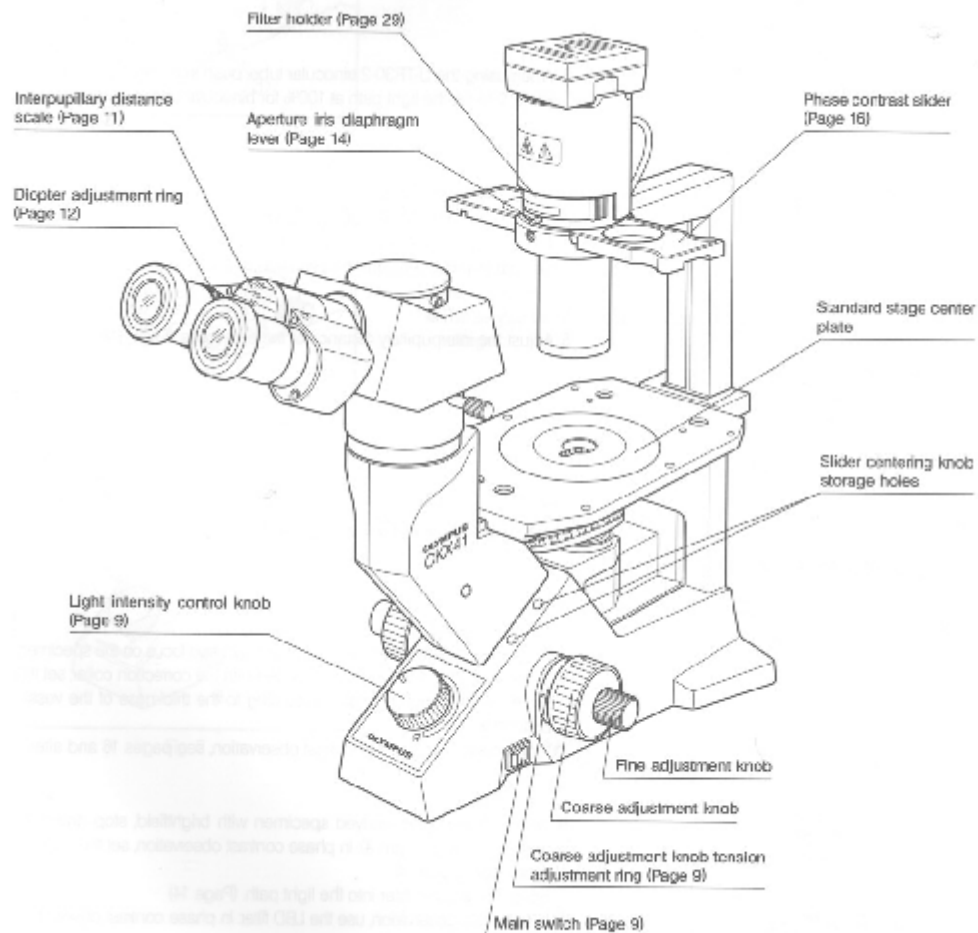
## 8. DOCUMENTATION

QS form - 009 Equipment decontamination certificate

QS form – 018 Preventative Maintenance, Inspection and service record

These forms can be found on the Loughborough University LEARN module for the Centre of Biological Engineering under QS-Form templates.

### ANNEX1: Schematic Diagram of CKX41 Microscope:



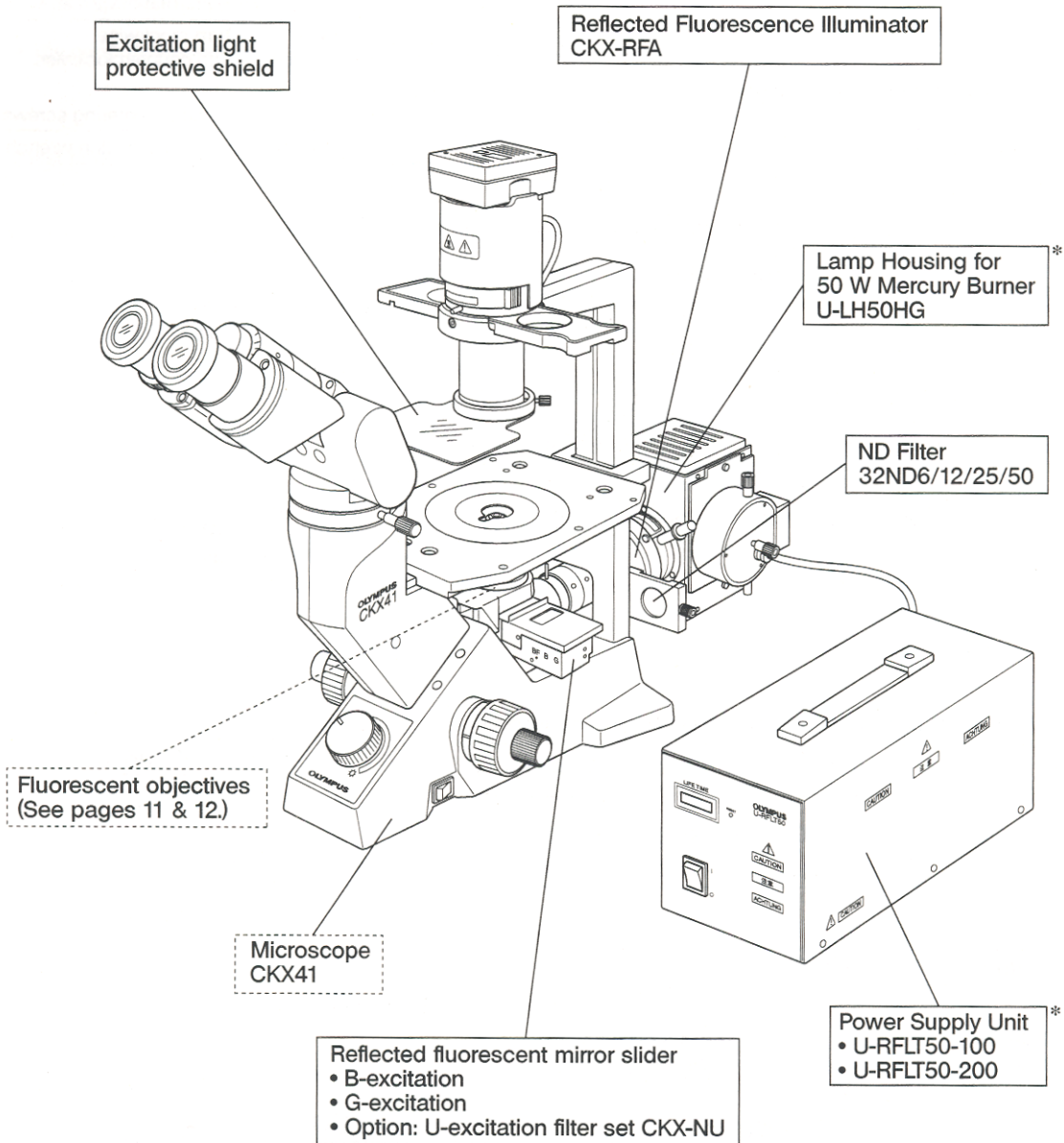
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ANNEX 2: Schematic Diagram of the Reflected Fluorescence System



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### **ANNEX 3: Schematic Diagram of Digital Camera System SOP Version History**

<b>Version Reviewed</b>	<b>Date Revised/ Reviewed</b>	<b>Revision Summary</b>	<b>New Version Number</b>
1.0	14.04.10 Y.Liu & C. Kavanagh	Annual Review – Minor editorial revisions. New version issue not required.	Not issued
1.0	23/02/2011 P.Hourd	Minor revision of scope and records sections following transfer of equipment to the T208b Tissue Engineering Laboratory, located in the Wolfson School	2.0
2.0	23/08/12 K.Sikand	Annual review carried out also alterations in layout to fit with lean SOP template. Next review in 2 years as per lean SOP system.	3.0
3.0	04/02/21 K.Sikand	Review carried out, minor revisions. Kept version number.	3.0

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