

Standard Operating Procedure

SOP080

Title: USE AND MAINTENANCE OF NIKON ECLIPSE TS100 INVERTED MICROSCOPE

Location: CBE LABORATORY H25

1. PURPOSE

To describe procedures for the safe use and maintenance of the Nikon Eclipse TS100 inverted microscope.

2. SCOPE

This SOP describes the routine procedures, associated hazards and risk mitigation involved in the use and maintenance of the Nikon Eclipse TS100 inverted microscope located in the animal cell culture room (H25) in the CBE.

The microscope can be used for transmitted light observations (phase contrast) 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.

3. RESPONSIBILITIES

3.1 The Operator:

- (i) Shall ensure familiarity with the equipment controls and requirements by reference to this SOP and the Operator and Maintenance Manuals, including operating instructions, record keeping and emergency shutdown procedures.
- (ii) 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 (refer to section 5).

3.2 The Responsible Person:

- (i) Shall schedule any service or preventative maintenance requirement with authorized service representatives. This must be coordinated with the Laboratory Manager.
- (ii) Shall ensure that the microscope has been suitably disinfected (see SOP 003) and a 'Decontamination Certificate' issued (see Section 6) before external maintenance and servicing is performed.
- (iii) Shall record all maintenance, servicing and repair work and file with the microscope equipment file.
- (iv) Shall ensure that all operators have been given appropriate information, instruction, training and supervision in the correct use and maintenance of the microscope.

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4. EQUIPMENT AND MATERIALS

- Nikon Eclipse TS100 inverted microscope with 10x and 40x lens and phase contrast slider
- Extra long working distance (ELWD) condenser for phase-contrast microscopy
- DS Camera Control Unit DS-L2
- Neutral cleaning solution (e.g. 70% IMS)
- Microscope slides
- Microscope cover slips

5. PROCEDURE

5.1 Microscope Operation

- i. Turn on the power to the microscope by pressing down on the rear-half of the power switch. Adjust the brightness of the view field with the brightness adjuster (clockwise to increase and anticlockwise to decrease).
- ii. Adjust the distance between the binocular eyepieces so that the left and right view fields overlap to form a single image.
- iii. Turn the dioptre adjustment ring on the right and left eyepieces to align the 0 line with the reference line.
- iv. Move the aperture diaphragm lever on the condenser to the right to fully open the aperture.
- v. Turn the revolving nose-piece to bring the 10x objective into the light path.

NOTE: Ensure that the revolving nose-piece is turned until it clicks.

- vi. Place the specimen on the stage. Adjust its position so that the centre of container comes under the optical path.
NOTE: Always localise your sample with 10x objectives before further observation using higher magnification.

NOTE: If using a 35 mm petri dish, always attach the supplied round-holed ring to the stage to prevent the dish from falling off.

- vii. Adjust the dioptre adjustment rings of the eyepieces according to the visual power of your left and right eyes. Once this adjustment is complete, full performance of the objective will be properly achieved, including their parfocality.

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- viii. If a phase slider is set, enter an empty hole into the optical path or remove the slider from the condenser.
- ix. Place the 10x objective in the optical path. Turn the coarse and then the fine focus knobs to bring the specimen into focus.
- x. Place the 40x objective in the optical path and turn the focus knobs to bring the specimen in focus.
- xi. Place the 10x objective in the optical path again. Look through the right eyepiece with your right eye and without touching the focus knobs turn the right dioptre adjustment ring to bring the specimen into focus.
- xii. Look through the left eyepiece with your left eye and without touching the focus knobs turn the left dioptre adjustment ring to bring the specimen into focus.
- xiii. Repeat steps (x) through (xii).
- xiv. If a phase slider was removed, return it to its original position.
- xv. Once specimen has been viewed, remove the sample and press down the front-half of the power switch to turn off the lamp. After the lamp house cools, cover the microscope with the vinyl cover so that it does not gather dust.

5.2 Phase Contrast Microscopy

- i. Attach the extra long working distance (ELWD) condenser to the illumination pillar.
- ii. Insert the phase slider into the condenser.

NOTE: If you are planning to use a Ph2 objective, attach the annular diaphragm Ph2 to the slider in advance.

- iii. Move the aperture diaphragm lever on the condenser to the rightmost position to fully open the aperture. Always fully open the aperture diaphragm for phase contrast microscopy. If the aperture diaphragm is closed, it will obstruct the annular diaphragm and the phase contrast effects cannot be obtained.
- iv. Centre the annular diaphragm so that it aligns with the phase ring of the objective.

NOTE: Only centre the annular diaphragm when using a phase slider which can be centred. Perform this adjustment with the centre of the container in the optical path. Avoid the periphery of the container.

- v. Place the 10x phase contrast objective (Ph1) in the optical path.

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- vi. Slider the phase slider to place the Ph1 annular diaphragm in the optical path.
- vii. Taking care not to alter the position of the dioptre adjustment ring, remove one eyepiece from the eyepiece tube and insert a centring telescope in its place.
- viii. Holding down the flange of the centring telescope, turn the eyepiece of the centring telescope and focus on the objective phase ring.
- ix. Insert two hexagonal screwdrivers into the centring holes on the phase slider and while looking through the centring telescope, centre the Ph1 annular diaphragm image so that it aligns with the phase ring.
- x. If you are using an objective with a Ph code other than Ph1, place that objective and the annular diaphragm having the same Ph code as that objective in the optical path and centre the annular diaphragm in the same manner as described above.
- xi. Select the desired phase contrast objective by turning the revolving nosepiece and place the phase contrast objective of the desired magnification in the optical path.
- xii. Place the annular diaphragm having the same Ph code as the objective in the optical path and observe. Since optical conditions worsen by the difference in the thickness of the container depending on the location, the centre of the annular diaphragm may become displaced. If the contrast suddenly worsens, check the condition of the setup and, if necessary, centre the annular diaphragm again.
- xiii. If the objective has a correction ring, adjust the ring according to the thickness of the container.

5.3 Waste Disposal

- i. Biological waste or mixed chemical/biological waste generated through the usage of the microscope should be disposed of according to SOP003 "Disposal of Biological Waste".
- ii. Microscope slides must be disposed of in sharp containers as instructed by SOP003 "Disposal of Biological (Healthcare) Waste".
- iii. Trypan Blue, used for cell counting, must be disposed of according to SOP029 "Safe handling and disposal of Trypan Blue". Small volumes (typically less than 50 uL of solution at 0.4%) of Trypan Blue, retained under microscope cover slips, should be washed into the sink followed by copious amounts of water.

5.4 Maintenance Procedure

5.4.1 General maintenance of microscope and camera control unit

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- i. If the lens becomes contaminated with any substance, contact the Responsible Person 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).

CAUTION: Do not spray 70% IMS directly onto camera screen surface, spray 70% IMS onto a tissue and wipe gently across screen. Ensure that no liquid enters the inner surface of the screen.

- iii. Clean non-glass microscope components using a lint-free, soft cloth moistened with a diluted neutral detergent.
- iv. Do not disassemble any part of the microscope or the camera control unit. This could result in malfunction or reduced performance.

CAUTION: Do not disassemble or dismount any part of the system without consulting the Responsible Person.

- v. When the microscope is not in use, reduce the brightness, switch off both the main switch and the power supply, and keep it covered with a dust cover.

NOTE: If the bulb is replaced, fill in the maintenance record and inform the Responsible Person.

5.4.2 Microscope or camera control unit malfunction

- i. Inform the Responsible Person if any part of the equipment fails or malfunctions. With permission of the Responsible Person the user should consult the Operator Instruction Manuals for fault finding and troubleshooting procedures.
- ii. Record all problems and corrective actions in the maintenance log (Section 6).
- 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 Responsible Person.
 - Attach a "Do Not Use" notice.

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- 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 by the School/Building/Unit Safety Co-ordinator. Section 6 states where the decontamination certificates can be found.
- Ensure that the scheduling of any external servicing/maintenance is coordinated with the Laboratory Manager.

5.5 Procedures for using the DS Camera Control Unit DS-L2

NOTE: Refer to the operator manual for more detailed instructions.

5.5.1 Viewing samples and saving images to USB

- i. To turn on the camera display, begin by pressing the power switch located on the bottom right hand corner of the display.
- ii. If the images are to be stored on a USB storage device, insert the USB device into a free USB drive slot located at the top right-hand side of the camera display.
- iii. To view the sample image on the camera screen as opposed to through binocular view on the microscope, turn the microscope dial located to the right of the microscope eyepiece from "bino" to "photo".
- iv. Bring the image to focus using the appropriate objective and focus knobs.
- v. Once the image is in focus, press the "Capture" button to save a copy of the image to the USB storage device.
- vi. The image has now been stored. To turn off the camera display, begin by switching the view from the display back to the microscope by turning the microscope dial from "photo" to "bino".
- vii. Press the power switch to turn off the camera display.
- viii. The USB storage device can now be safely removed from the camera display unit.

5.5.2 Accessing the menu screen and specifying save folder

- i. To access the main menu, right-click the mouse on the upper third of the screen.
- ii. To access the shortcut menu, right-click on the lower third of the screen.

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- iii. To change the date and time, open the setup menu by pressing “[SETUP]” tab from the main menu.
- iv. Select the “Additional” tab on the setup menu and under the “Additional Settings” section, adjust the date and time.
- v. To specify the Save Folder and File Names, select the “File Set” tab under the setup menu and name the folder and file where you want to save captured images.
- vi. To specify a folder name, input the desired name in the “[Media Dir]” field, using a maximum of eight characters. If the auto box is selected, a folder will be automatically created based on the save date.
- vii. To specify a file name, specify the first string of up to four characters. This string will be followed by an automatically assigned serial number, which together form the name of the save file. If the auto box is selected, the name of the file name will consist of eight characters representing the day, hours, minutes and seconds.

5.5.3 Adjusting photography options

- i. To adjust the white balance, select the “[CAM]” tab on the main menu and observe an evenly white subject such as a transparent part of the preparation. Then click the “[WB]” button on the “CMP.” row of options, or click the “WB” button on the shortcut menu.
- ii. To set the file format and size, select the “[REC]” tab on the main menu and adjust the file format and file size at the bottom of the menu screen.
- iii. To adjust the image to allow for different biological specimens, select the “[CAM]” tab on the main menu and choose from one of five different “biological specimen scene modes” located at the bottom of the menu. The different scene options are listed below:
 - (i) **[DF/FL]:** Dark field photography/fluorescent photography – use this mode to photograph a dark subject. The camera operability for position or focus is improved by increasing the camera’s sensitivity. High-resolution images can be obtained from the photographed data.
 - (ii) **[BF]:** Bright field photography – intended for general stained specimens. In the case of EVG staining, a good result may be obtained by setting R of the colour balance adjustment to 94.
 - (iii) **[DIC/PH]:** Contrast is enhanced for differential interference and phase contrast photography purposes.
 - (iv) **[HE]:** Provided for photographing of HE stained specimens. This mode is optimised for colour reproduction specific to HE.

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- (v) **[ELA]:** Provided for photographing of ELA (Enzyme labelled antibody method). This mode is optimised for colour reproduction specific to DAB.
- iv. To adjust the brightness, select the “[CAM]” tab on the main menu and click the “+0.3” button on the “CMP.” row. Alternatively, click the exposure compensation button on the shortcut menu (first button on the left).
- v. To review a photographed image, click the “[VIEW]” tab on the main menu and select the desired filename. Alternatively, click the data reproduction button on the shortcut menu (last button on the right).
- vi. To display a measurement scale, select the “[TOOL]” tab on the main menu and select the “Scale” button. A scale appears, allowing you to measure the approximate size of the subject. To adjust the scale, select options “m1” to “m7” located at the bottom of the “[VIEW]” menu. Select the “Scale” button to display the scale on the lower right of the screen.
- vii. To change the scale length and colour, select the “Scale” button on the “[TOOL]” menu, move the cursor over the displayed scale and left-click the mouse. A menu for changing the scale length and colour appears. To change the length, enter the desired value using the onscreen keyboard and then press the “Ent” button. To change the colour, select one from the six colours, red, green, yellow, blue, white and black.
- viii. To render the scale in the saved image, select the “[TOOL]” tab on the main menu, then select the “[REG>>]” menu and then select the “[OTHER]” menu. In the “[Paste to Image]” section, add a checkmark to “[Scale]”.

5.6 Health and Safety procedures/precautions.

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 before and 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 will become extremely hot during operation.

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- (iv) Ensure that the microscope is covered with the designated dust cover when not in use.

CAUTION: Switch off both the main switch and the power supply before covering the microscope. Ensure that the lamp housing for the microscope is 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 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 there is ample free space around the lamp housing (especially above and below). The lamp housing parts get extremely hot during operation.

- (vii) Always wear appropriate PPE i.e. lab coat and gloves for handling specimens for observation with the microscope.

- (viii) If using a hazardous chemical agent, such as Trypan Blue, it is important to ensure that the chemical has a relevant COSHH form and that any specimen contaminated with the chemical agent is handled and disposed of according to the COSHH form and COP. Special waste procedures may be required for waste containing hazardous chemical agents, refer to SOP003 "Disposal of Biological Waste". For Trypan Blue specifically, please refer to SOP029 "Safe Handling and Disposal of Trypan Blue".

6. 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.

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SOP Version History

Version Reviewed	Date Revised/ Reviewed	Revision Summary	New Version Number
001	17/08/10 Revised by QR	1. Added section 7.5: "Procedures for using the DS Camera Control Unit DS-L2" 2. Amended section 7.4 (General Maintenance) to include maintenance of camera control unit 3. Reformat of Maintenance Log	002
002	04/02/21 Revised by KS	1. Fitted SOP to lean template. 2. Removed forms from SOP and added reference to LEARN system where the templates can be found. 3. New version number given to SOP.	003

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