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Title: **Use and maintenance of the Zeiss Axiovert 40 CFL microscope in Room 248O (Microscope Room)**

Version: **v2**

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Valid to:	25/09/2023

SOP History		
Number	Date	Reason for Change
v1	26/09/2013	Original
v2	26/09/2018	Update and change of author

1.0 Purpose –
The purpose of this SOP is to outline the principles of the routine use of the Zeiss Axiovert 40 CFL microscope in Laboratory 248O (Microscope Room) at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies to routine use and maintenance of the Zeiss Axiovert 40 CFL microscope within the SASoM.

3.0 Responsibilities –
It is the responsibility of all users of the Zeiss Axiovert 40 CFL microscope within the SASoM to comply with this SOP.

4.0 Procedure –
Users new to handling microscopes should undergo basic training in how to use a microscope.

A booking sheet for this microscope is present and, if possible, usage should be noted in advance (Name and group initials). This will

1. Avoid clashes with other users and
2. Maximise efficient use of the microscope and its light bulbs.

- Once finished, check with other signed up users if light bulbs should be left on.

The Zeiss Axiovert 40 CFL microscope is an inverted microscope, able to take bright field, phase contrast and fluorescent images.

1. Always maintain the microscope in a good condition and wipe away any spills on the stage immediately with water, then 70% ethanol using white tissue.
2. ALWAYS use lens tissue if lenses require to be cleaned.
    a. Dampen lens tissue with 70% ethanol or isopropanol
    b. Gently wipe the lens of the objective without applying pressure. DO NOT rub!
3. Take care not to damage the objective lenses under the stage.
    a. Always lower the objective turret using the coarse control before turning it to change the objective.
4. Always switch off the microscope after use.
    a. Cover the microscope when not in use.
    b. ALWAYS ensure the lamp is SWITCHED OFF before covering.
5. Use the mechanical stage control with the mounting frame fitted for microscope slides and small (24 - 68mm) culture dishes.
    a. Adjust the 2 holders in the mounting frame by pressing the 2 buttons on either side of the sliders and bringing them into a position which will accommodate the microscope slide or dish to be observed.
    b. Move the specimen using the X-Y control knobs at the right of the stage control
    c. The mounting frame can be removed by sliding it towards the back in order to accommodate larger dishes or plates. ALWAYS lower the objective before removing the mounting frame!

<u>Bright field and phase contrast:</u>

1. Switch on the microscope lamp at the green switch on the right-hand side of the microscope.
2. Place either microscope slides or cultured cells (flasks/ plates) on the stage.
3. Adjust the phase contrast slider on the top of the microscope below the light source for phase or bright field.
    a. The type of phase ring to use, Ph1 (left) or Ph 2 (right), is indicated on the objective.
    b. The middle section of the slider is for bright field microscopy.
4. Use the appropriate objective lens and focus using the dials at each side of the microscope.
    a. Look down the eyepieces with both eyes (not wearing glasses) and adjust their distance until the field of view can be seen as one circle.
    b. Use the coarse control (outer part of the dial) to bring the objective NEAR the slide or dish.
    c. Use the fine control (inner part of the dial) in order to optimise the focus.
    d. If necessary, adjust the dioptre at one of the eyepieces in order to compensate for differences in vision between eyes.

Image acquisition uses the CCD camera and computer program (AxioVision 4.1) to capture digital images.

Fluorescence:
In order to take fluorescence images, the HBO 50 mercury lamp power source should be switched on.

- This is a high energy lamp (a 50-watt high-pressure mercury plasma arc-discharge lamp). It should be run for at least **30 minutes** before being turned off and also left for 30 minutes before being turned on again.
- Lamp usage (name/ group initials and time) MUST be logged in the sign up book and the lamp hours, given in the display at the front of the lamp recorded.
    - The mercury lamp has a maximum lifetime of 200 hours and should be used beyond this in order to guarantee user safety.
    - Generally, the total number of ignitions should not exceed one half of the total number of hours: Maximal 100 ignitions are recommended.

1. Switch on the mercury lamp.
2. Open the fluorescence light shutter on the right-hand side towards the back of the microscope.
3. Place the appropriate filter block for the desired wavelength of light:
    a. left: UV light for blue fluorescence/DAPI
    b. middle: blue/turquoise light for green fluorescence/ FITC
    c. right: green light for red fluorescence/ TRITC

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5.0 Personal protection –
Howie coat must be worn at all times.

6.0 Training –
All users have to be trained before using the Instrument by a designated person.

7.0 Related documents –

RISK ASSESSMENTS:	RA/GEN/016
	RA/COSHH/OO4
SOPs:	SASoM-EQUIP-042

8.0 Approval and sign off –

Author:

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Signature: Date:

Management Approval:

Name: Mary Wilson

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Signature: Date:

QA release by:

Name: Alex MacLellan

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