

TES10 - Fluorescence Microscopy for Fiber Examination

Scope

This document addresses the methods for setting up the microscope and completing individual fiber examinations using the fluorescence microscope. This procedure applies to fiber samples that have been previously removed from evidentiary items and have been mounted on glass microscope slides using the Evidence Handling Procedures.

Safety Precautions

One must not look directly at ultraviolet radiation as it will damage the eye.

Materials Required

- Nikon Eclipse E600 polarized light with a Y-FL Epi-fl attachment (or similar) with excitation filters containing the following wavelength ranges: 330-380 nm, 380-420 nm, 450-480 nm and 510-560 nm.

Procedure

1. Set up the microscope for proper Kohler illumination and alignment. Refer to TES09.
2. Turn on the light source for the microscope (switch is located on either side or back of the base of the microscope).
3. Turn on the light source for the fluorescence capabilities (power source box located next to the microscope).
 - a. Turn on the power (“Power” light should appear green).
 - b. Press and hold the “Ignition” button until orange, “Lam Ready” light remains on.
4. View the samples under plane polarized light. Ensure the shutter switch is closed and you are using the 380-420 nm excitation filter.
5. Turn off the microscope light source or switch to epi illumination (switch on the upper back side of microscope) and open the shutter switch to view any possible fluorescence.
6. Select the desired excitation filter by moving the horizontal, four-filter linear slider.
7. There is a filter block at each lever position that consists of three types of optical components: an excitation filter (EX filter), a barrier filter (BA filter) and a dichroic mirror (DM). The barrier filter limits fluorescence to wavelengths greater than the barrier filter.
8. The filter cubes placed at each lever position can vary depending on the microscope set up.

Lever Position	Wave length of excitation filter	Barrier filter
1	510-560 nm	590 nm
2	380-420nm	450 nm
3	450-480 nm	515 nm
4	330-380 nm	435-485 nm

9. Document observations (color and intensity) on the fiber comparison form at each excitation range.
10. Compare visual observations made at each of the different excitation ranges for the known and unknown samples.

Comments

Do not leave samples exposed to the ultraviolet radiation as it can degrade the sample.

References

- Chambers, William, Thomas J. Fellers and Michael W. Davidson. "Steromicroscopy Epi-Fluorescence Illumination". Nikon MicroscopyU. Nikon Instruments, Inc., 2000. Web.
<http://www.microscopyu.com/articles/stereomicroscopy/stereofluorescence.html>
- Grieve, Michael and James Robertson. "10" Forensic Examination of Fibres/Edited by James Robertson and Michael Grieve. 2nd ed. London: Taylor & Francis, 1999. 275-277.
- Nikon Y-FL EPI-Fluorescence Attachment Instructions (Instruction manual for the Nikon Eclipse E 600 polarized light microscope with a Y-FL Epi-fl attachment).
- Ploem, J.S; Tanke, H.J., Introduction to Fluorescence Microscopy, Oxford Science Publications, 1987, Chapters 1 through 3.