8.3 Fluorescence Microscope & Comparison Fluorescence Microscope

8.3.1 Introduction and Theory

Fluorescence is the emission of light by a substance that has absorbed light or other electromagnetic radiation of a different wavelength. In most cases, emitted light has a longer wavelength, and therefore lower energy, than the absorbed radiation. When organic or inorganic specimens absorb and subsequently reradiate light the process is typically a result of fluorescence or phosphorescence. Fluorescence emission is nearly simultaneous with the absorption of the excitation light as the time delay between photon absorption and emission is typically less than a microsecond. George Gabriel Stokes coined the term "fluorescence" in the middle of the 19th century when he observed the fluorescence emission almost always occurred at a longer wavelength than that of the excitation light.

Fluorescence may arise from an item itself (e.g. such as a fiber itself) or from dyes and other additives.

Fluorescence (or lack thereof) can often be used to compare a questioned sample to a known sample.

Terminology:

Beam splitter (B/S) wavelength (example the "D" cube 455nm) = The wavelength of light that is passed along (allowed through) after going through the beam splitter

Emission wavelength (example the "D" cube 470nm Longpath (LP)) = Via a further filter, anything above this wavelength goes through to be observed by the eye

Excitation wavelength (example the "D" cube 355-425nm) = The range of wavelengths of light that is allowed through the first filter (to the sample)

Fluorescence = The emission of light of a certain wavelength by an object when excited by light of a shorter wavelength (higher energy).

Filter Information:

Fluorescence Cube	Color	Excitation Wavelength	Beam Splitter Wavelength	Emission Wavelength
D	violet	355-425 nm	455 nm	470 nm
H3	blue	420-490 nm	510 nm	515 nm
I3	blue	450-490 nm	510 nm	515 nm
N2.1	green	515-560 nm	580 nm	590 nm

8.3.2 Safety & Bulb Information

The fluorescence microscopes are equipped with high pressure bulb light sources and certain precautions should be observed to prolong bulb life, lessen the danger of bulb explosion and assure high energy output. Mercury bulbs have an average operating life of 200 hours. Hours higher than that can weaken the bulb and make it more prone to exploding.

The bulb should remain on (lit) for a minimum of 20 minutes. Reportedly, it takes approximately 20 minutes for the mercury to vaporize completely. Turning the bulb off and on for shorter increments can lead to mercury deposits on the inside surface of the bulb.

If you plan to use the scope several times during the day, it is better to leave the bulb on continuously than to turn it off and relight it.

8.3.3 Minimum Standards and Controls

A known fluorescing sample should be checked to ensure that the fluorescence microscope is working properly prior to viewing any samples.

For comparisons using the fluorescence scope, a known "matching" fluorescing sample (ex: fibers from the same source) should be placed on each scope and examined with each fluorescence range to determine light and fluorescence balance.

If measurements are made with an ocular scale, please refer to *Trace Verification Standards 12.2 Microscopic Ocular Unit Calculation*.

8.3.4 Operation

Samples should be mounted in a low fluorescent or non-fluorescent medium.

- Turn on the main switch of the power supply unit. The bulb arc will intensify and stabilize in 2-3 minutes. The scope is now ready for fluorescence use. The bulb should remain on (lit) for a minimum of 20 minutes. If you plan to use the scope several times during the day, it is better to leave the bulb on continuously than to turn it off and relight it.

Note: To avoid sampled bleaching, close the fluorescence light shutters whenever you are not observing the specimen(s).

Note: Ensure that the wave plate is not in the light path as the fluorescent light can also damage its qualities.

- 2. Setup microscope for typical brightfield imaging.

- 3. Rotate the fluorescence cube holder until the filter cube is utilized. Examine the sample(s) with each cube.

Information to record: The intensity (e.g. - none, low, moderate, high) and the color of the fluorescence for each cube

A Fluorescence Microscopy Worksheet (Trace Appendix 8.3 A) can be utilized if desired.

8.3.5 References

Standard Guide for the Microscopic Examination of Textile Fibers; ASTM Method E2228 (current version); ASTM International, 100 Barr Harbor Drive PO Box C700, West Conshohocken, PA

Spring, Kenneth R.; Fluorescence Microscopy, Encyclopedia of Optical Engineering DOI: 10.108/E-EOE 120009628; 2003 Marcel Dekker, Inc.