I. Scope:

This method describes the procedure for setup and use of the reflected light fluorescence attachment on the PLM microscopes. Fluorescence microscopy is utilized for detecting and characterizing auto fluorescing substances in materials such as pigments in paints and for comparing fibers that have fluorescing dyes or optical brighteners. The procedure below is for both the mercury and xenon lamps.

II. References:

1. Olympus BX-FLA Reflected Light Fluorescence Attachment Instruction Manual.
2. Leica DML Instruction Manual – Incident Light Chapter
3. SWGMAT Fiber Guidelines - http://www.swgmat.org/fiber.htm
4. SWGMAT Paint Guidelines – http://www.swgmat.org/paint.htm
5. *ASTM Annual Book of Standards: (Vol. 7.01-7.02). Textiles.* American Society for Testing and Materials, West Conshohocken, Pennsylvania, 1996.
6. Forensic Examination of Fibres by James Robertson and Michael Grieve

Validation

Fluorescence microscopy is a well known and scientifically accepted method for the analysis and comparison of fluorescing materials in many types of trace evidence. Relevant examples of the broad nature of the method and related literature can be found in Section II (References).

III. Apparatus/Reagents:

1) Reflected Light Fluorescence Attachment
2) Fluorescent Cubes

Standards

While there is no required standard for the use of this instrument, a sample having known fluorescence may be examined to ensure the instrument is working properly.

IV. Safety Precautions:

1) Standard laboratory safety procedures will be followed along with those recommended in manufacturers' instruction manuals.

2) Gloves and safety glasses should be worn when handling the lamp.
3) Never look directly at the UV light source.
4) Allow the lamp housing and lamp to cool before changing a burned out lamp.

V. Procedures:

UV Lamp Alignment and Adjustment:

1) The alignment and adjustment should be conducted whenever the lamp is changed or whenever necessary before an examination.
2) Refer to manufacturer's instruction manual for specific instructions on the adjustment and alignment of the mercury or xenon lamps.

Warning: if using the mercury burner (lamp) the follow precautions should be followed:

-If the burner does not ignite, turn the main switch off once, and then repeat after 5 or 10 seconds.
-To avoid shortening the life of the burner, do not turn the burner off within 15 minutes of ignition.
-After turning the burner off, it cannot be re-ignited until the mercury vapor cools and condenses to liquid. Wait for about 10 minutes before restarting the burner.

Reflected Light Fluorescence Observations**:**

1) Close the vertical light path shutter and turn on the UV lamp (See warning above if using the mercury burner).
2) Using the transmitted quartz halogen light source, focus on the specimen using the desired objective.
3) Bring a suitable cube into the light path and turn off the transmitted light source.
4) Open the vertical light shutter and re-focus on the specimen, if necessary.
5) Adjust the collector lens-focusing knob to where the brightness and evenness of the illumination in the field of view are at a maximum. Adjust the aperture diaphragm as needed.
6) The intensity (e.g. – none, low, moderate, high) and the color of the fluorescence should be noted.

VI. Quality Assurance/Quality Control:

1) Microscopes should be checked for proper set-up before the examination is conducted and re-adjusted as needed during the examination.
2) Microscope should be cleaned and adjusted regularly by a manufacturer's service representative per the service contract agreement.
3) There is no known error rate for this type of examination.