CHAPTER 4

FLUORESCENCE MICROSCOPY

4.1 THEORETICAL ASPECTS

4.1.1 The origin of fluorescence

Ultraviolet or visible light can cause electrons in the ground state to reach a higher energy state. The electrons undergo excitation. After losing some of their energy, these electrons can reach the ground state again through light emission. This photoluminescence phenomenon is called fluorescence. The pathway leading to fluorescence is visualized schematically in **[Fig 4-1]**.

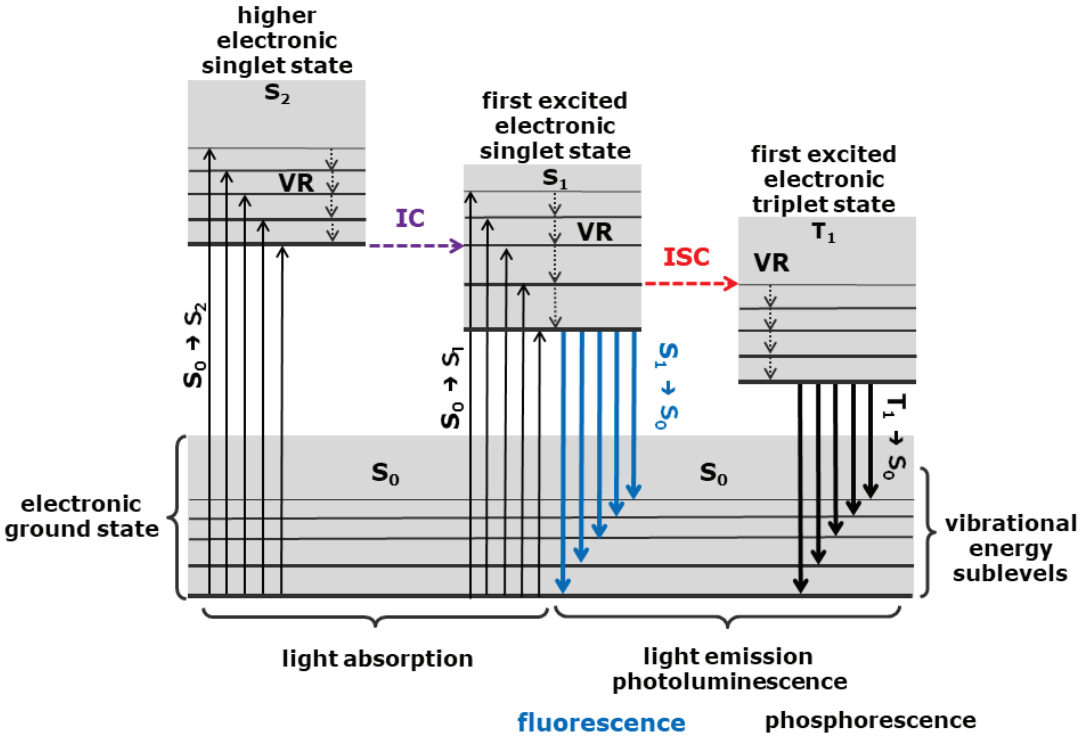


Fig. 4-1 Jablonski energy diagram depicting the pathway to fluorescence.

Typically, light absorption happens very fast within a femtosecond (10^{-15} s). This excitation to a higher electronic state is followed by two mechanisms that both occur within 10^{-12} s. One of these is vibrational relaxation (indicated with VR), in which a lower vibrational

A lamp socket with centring screws is available to optimize the excitation and obtain a homogeneous illumination field. The alignment of these lamps is a tedious procedure. Also, at the first operation, these lamps should be allowed to burn in for several hours to avoid flickering. If the lamp is only lit shortly, a second use could produce another small crater in the electrodes, which could provoke flickering. Mercury vapour sources are becoming obsolete because the breaking of the light bulb spills mercury which constitutes a health risk.

Xenon arc lamps (or XBO) consist of a quartz bulb with xenon gas at extremely high pressure. If a lamp is dropped or explodes, this causes a high-speed projection of glass fragments. Because of these safety issues, a protective facial mask is recommended whenever changing or adjusting xenon lamps. Another issue is that these high-intensity lamps produce harmful ozone, and an air extraction system should be provided.

Metal halide sources (MHS or HMI) have several advantages over other discharge arcs. These are small mercury arc lamps with metal halide electrodes. The bulb is pre-centred in a lamp housing and coupled to the microscope via a liquid light guide, requiring no alignment at all. The metal halide source is more efficient, produces highly stable light and has a broader spectrum than the regular mercury vapour source. Moreover, the metal halide source has a long lifetime, up to 10’000 operation hours.

LED illumination sources are the newest environment-friendly technology, as opposed to mercury lamp systems. Some of the LED systems can be operated for excitation wavelengths that cover the near-UV and the visible light, and the source can be used in multichannel illumination mode. Whereas the other excitation sources require some warming-up time, a LED illumination can be used instantaneously. These sources have a very long lifetime, up to 25’000 hours of use.

4.2.4 Fluorescence cubes

A fluorescence cube is a block containing three filters, each with a specific function. A drawing of the type of fluorescence cube used for fibre work is shown in **[Fig. 4-5]**.

The **excitation filter** (EX) is a bandpass filter (BP) that transmits only a limited wavelength range. This light arrives at a **dichroic mirror** (DM), also called a beam splitter. This is mounted 45° oblique to the plane of the excitation filter. It does not transmit the excitation light but reflects it towards the sample. After interaction with the sample, the emitted fluorescence and a small portion of reflected excitation light arrive again at the dichroic mirror. This filter lets through all fluorescence but guides the reflected excitation

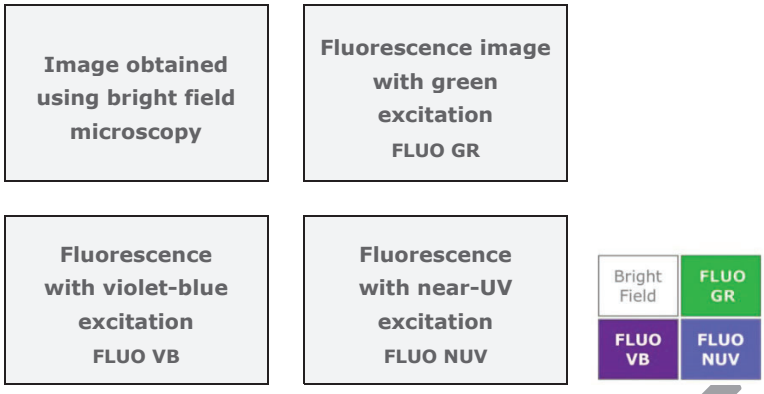
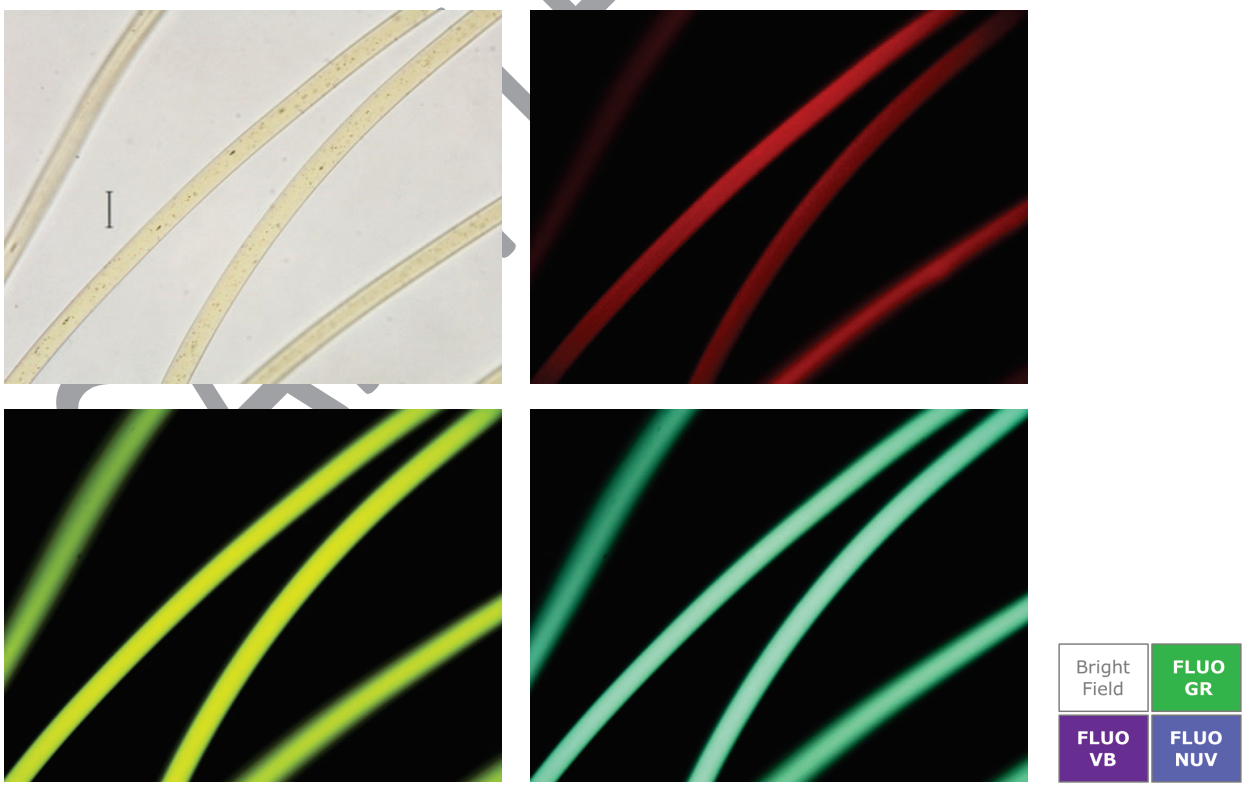
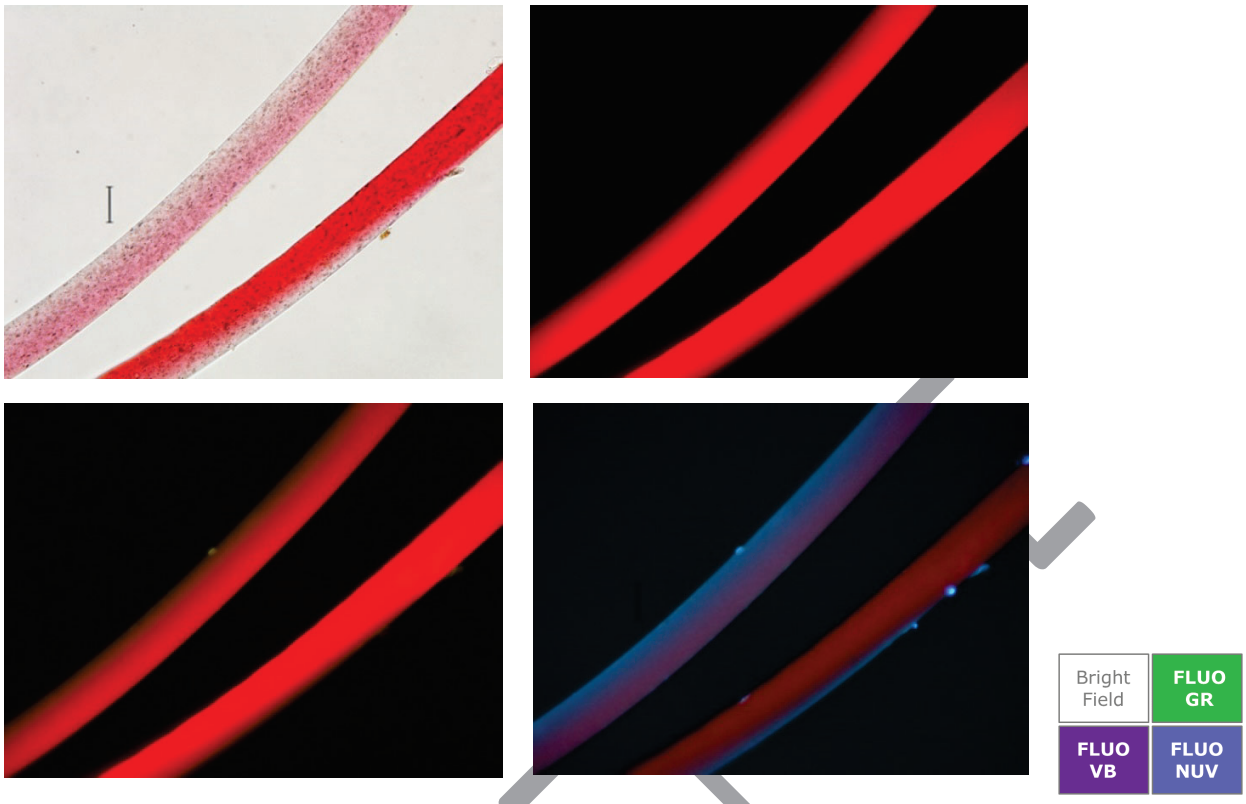


Fig. 4-8 Standard presentation of fluorescence images.

Fluorescence is usually observed at a total magnification of 400x. However, when examining a control sample, it is good practice to check the fluorescence at a lower magnification, for instance, at 50x or 100x. This allows one to verify whether these fibres exhibit an even fluorescence over the entire fibre length.



Ph. 4-1 Fluorescence in an evenly dyed yellow polyester fibre.



Ph. 4-6 Uneven fluorescence in a printed polyamide fibre.

4.4.5 Pigmentary prints

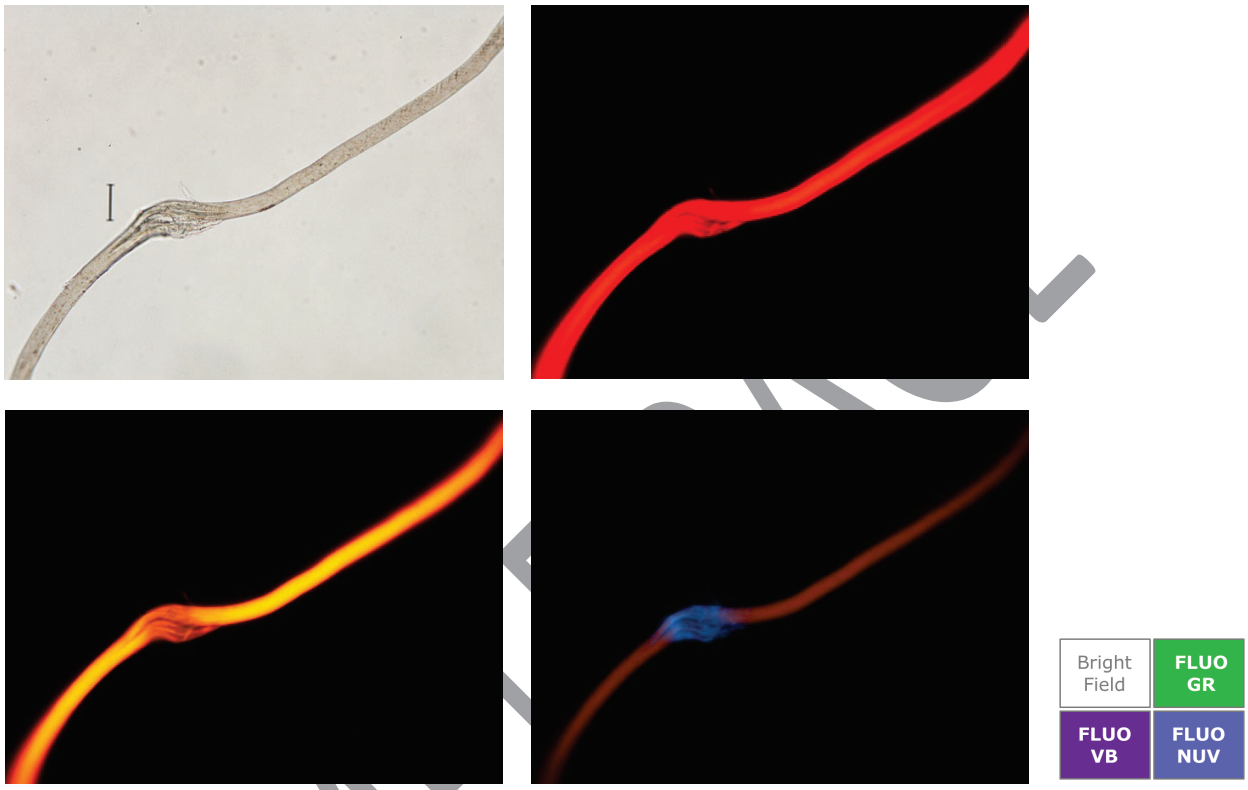
In some applications, pigmentary prints are used on man-made fibres. An example is given in **[Ph. 5-8]** of the next chapter. Here, the thick abrasive fibres found in scouring sponges contain a pigmented resin that has been applied to the fibre surface. The bright colour of these household sponges is due to these pigments. The scouring properties of these pads are due to abrasive particles present in the resin.

4.4.6 Inclusions

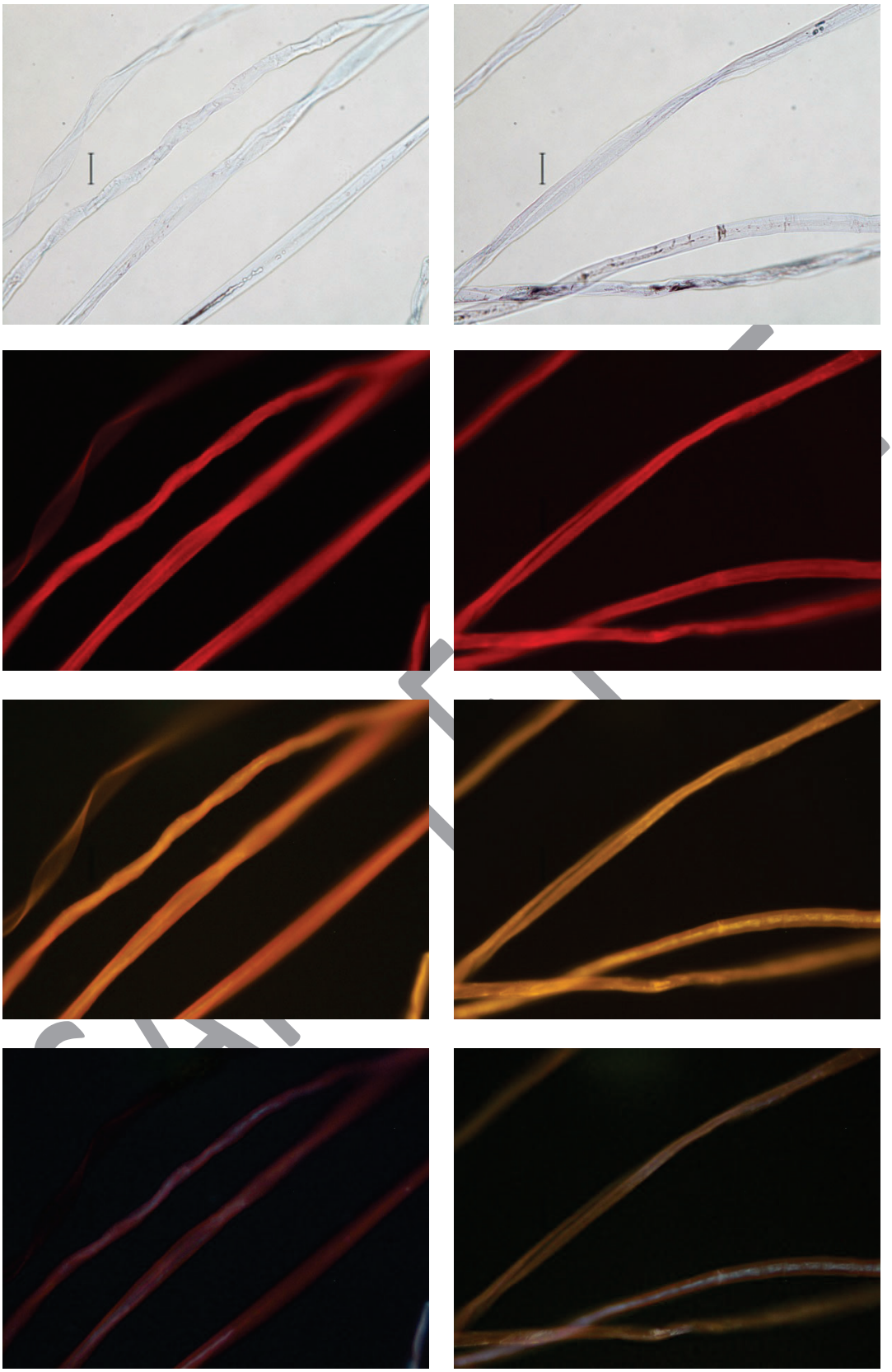
In some fibres, crystals have been added to obtain unique characteristics. For instance, "P1237 Footlights" by Du Pont company are trilobal polyamide fibres doped with zinc sulphide (ZnS) crystals of different sizes. These inclusions are easily visible in bright field microscopy because the transparent crystals are heavily outlined by fish eyes, as shown in **[Ph 4-7]**. The zinc sulphide crystals show a strong green fluorescence when excited with violet-blue light and a strong blue fluorescence when excited with near-UV.

4.4.9 Fibre damage

Damaged fibres may sometimes show a peculiar fluorescence. The fibre in **[Ph. 4-11]** shows **axial splitting** and strong fluorescence with violet-blue and green excitation. An aberrant fluorescence with near-UV excitation is observed in the damaged zone.

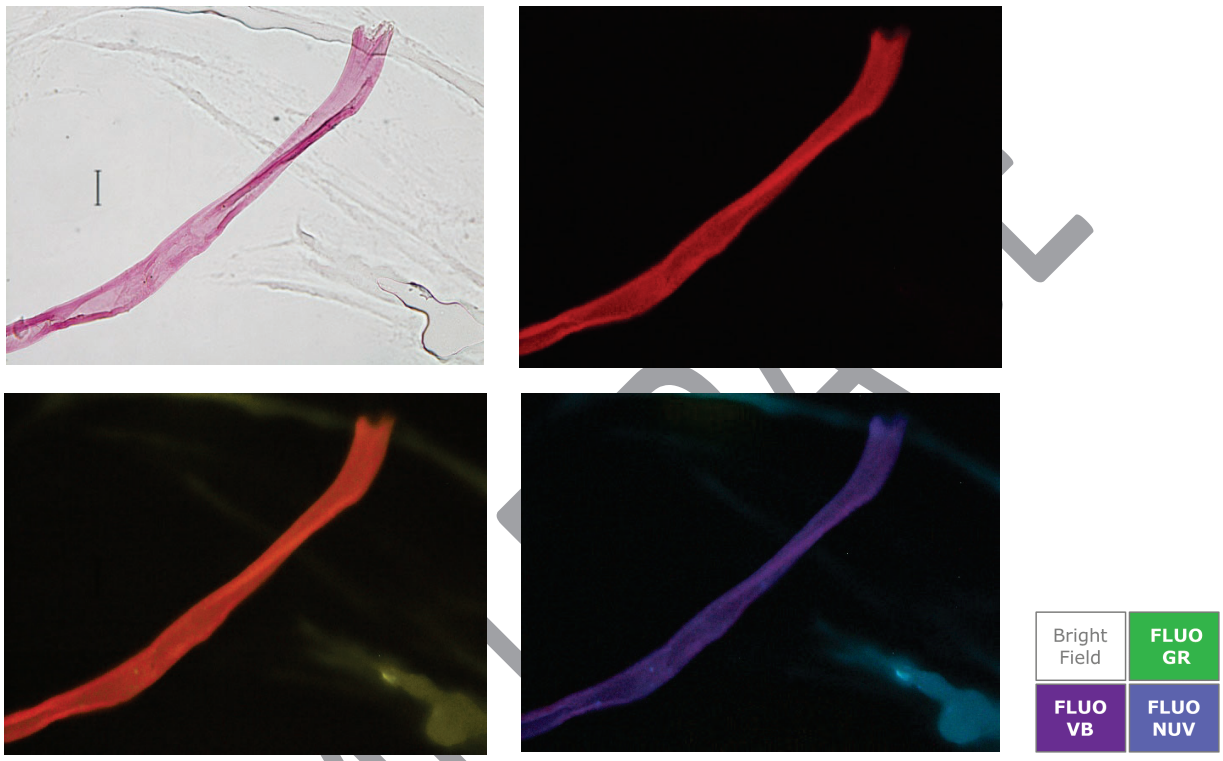


Ph. 4-11 Fluorescence in a damaged fibre.
400x magnification, I = 25 µm.



Ph. 4-14 Discrimination of fibre samples with fluorescence microscopy.
The two samples of faintly dyed blue cotton resemble each other using bright field microscopy. Different fluorescence colours are observed with VB and NUV excitation.

With near-UV excitation, sometimes a blueish instead of a black background is obtained. This blue glow is mainly due to the fluorescence caused by biological substances. If this fluorescence is still present after cleaning the microscope slide, little else can be done except remove and remount the sample after washing it thoroughly.



Ph. 4-15 Fluorescence of glue residues.
400x magnification, I = 25 µm.