

CHAPTER 5

COMPARISON MICROSCOPY

5.1 INSTRUMENTAL ASPECTS

5.1.1 The comparison microscope

Comparing fibre traces with control samples using a microscope can be a daunting task. The comparison microscope can facilitate the microscopic examination, especially when it comes down to describing the fibre morphology.

The comparison microscope consists of two identical microscopes interconnected with an optical bridge. This instrument enables one to observe two samples at the same time, under similar illumination. The bridge allows for a split observation field in which the left part contains the image formed by the left microscope, whereas the right part contains the image formed by the right microscope. People often say incorrectly that with comparison microscopy, one observes both samples in the same field of view. Actually, the comparison microscope combines the field of view of both microscopes. Therefore, it is of utmost importance that all optical components and filters used in the two microscopes match perfectly.

In this work, the control sample called the **known fibre sample** (K) is always put on the left microscope's object table. The fibre trace or **questioned fibre sample** (Q) is put on the right object table. As a reminder of this arbitrary convention, the caption of fibre images shown in this chapter indicates (K | Q).

A thorough comparison of the two samples is then performed at a suitable magnification. Usually, a total magnification of 400x is preferred, but 200x may be more appropriate for thicker fibres.

The comparison microscope has one main advantage: the **side-by-side observation** of the known fibre sample and the questioned fibre sample allows for direct and detailed observation of all morphological features. For instance, the number of delustrants, their size and distribution throughout the fibre are all characteristics used in the comparison process. Suppose the two samples were viewed one after the other on a regular microscope. In that case, it could be challenging for the microscopist to remember the

5.2 PRACTICAL ASPECTS

5.2.1 Background colour adjustment

For comparison microscopy, the difference in illumination of the two observation fields in bright field mode used to be critical. Due to the new designs of these instruments, these problems have been largely overcome. Still, the background adjustment remains a point of attention. In most instruments, colour correction is available on both microscopes.

First, a neutral background should be set on one of the microscopes using the colour correction buttons, shown in [Fig 5-3]. These engage blue, green or red colour filters to modify the light colour. After that, the colour correction buttons of this microscope are no longer touched. The final colour correction should now be made with the buttons on the other microscope. This procedure should ultimately lead to an adjusted background.

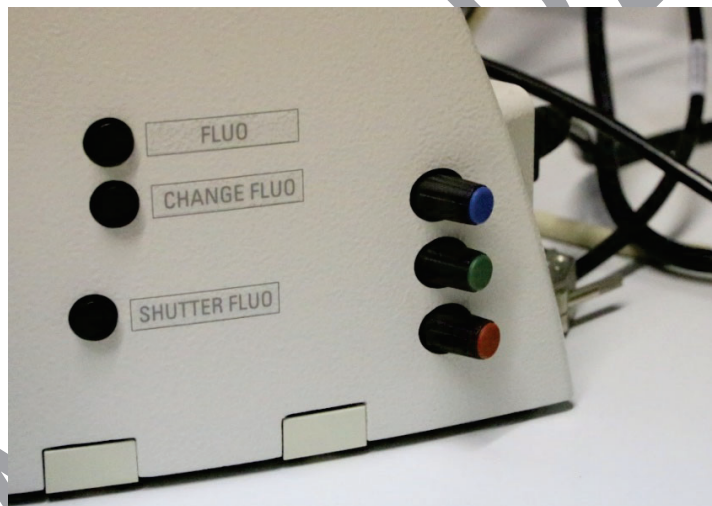
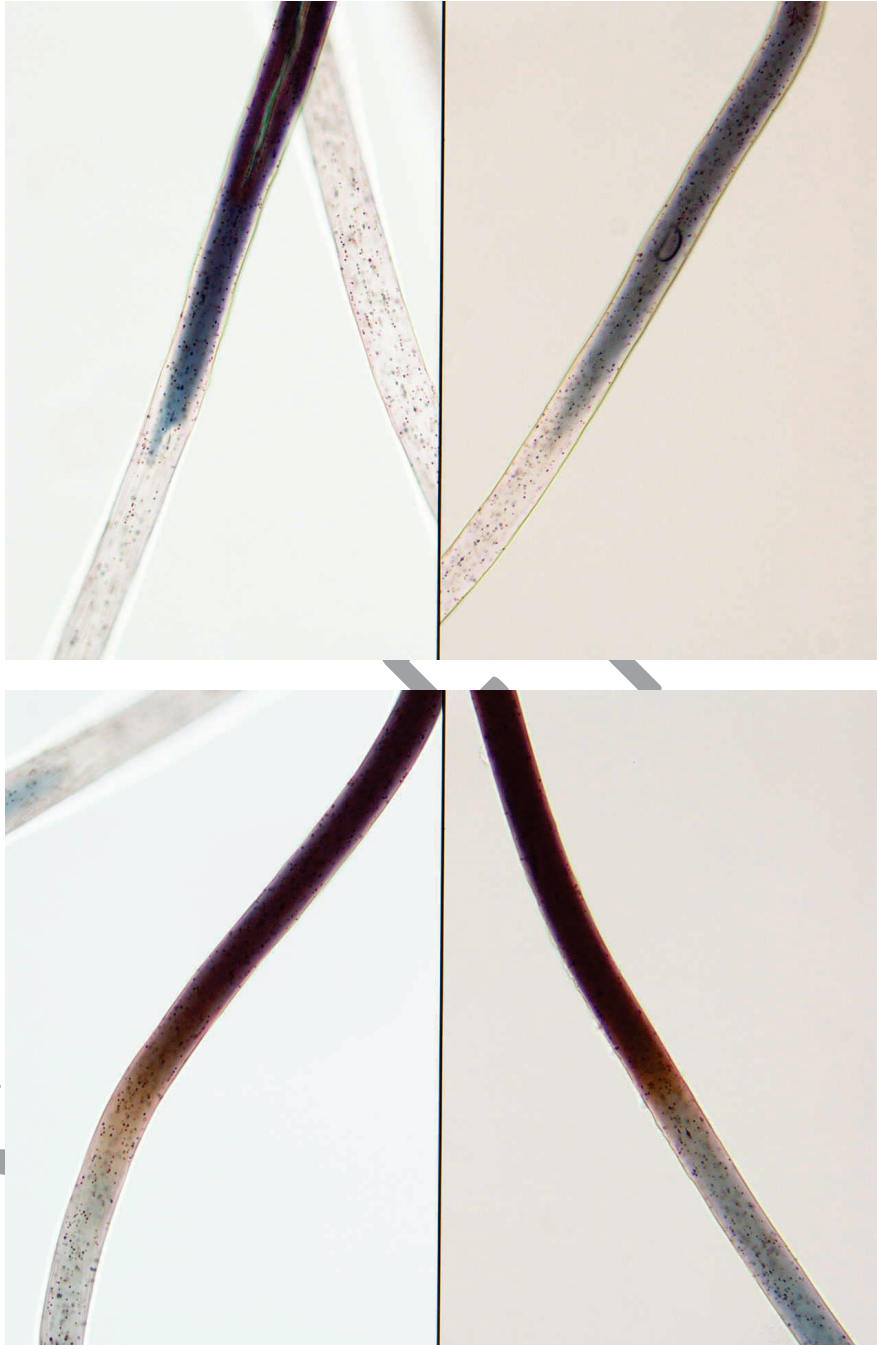


Fig. 5-3 Colour correction buttons.

Adjustment of the background colour can be quite tricky and time-consuming. If comparison microscopy is used routinely in the lab, the operators must be adequately trained in this procedure. If one is using the comparison microscope for observation, a compensated background is not that important. The human eye can correct for the colour difference in the two fields of view. However, colour compensation is critical if photomicrography is going to be used to document the comparison process.

[Ph. 5-3] illustrates the difference between a reasonably good and mediocre background compensation.

A more specific fibre comparison is shown in [Ph. 5-6]. The known fibres (K) are dip-dyed acrylics that originate from a fake fur collar. This fibre type has differently dyed zones, in shades of blue (upper image) and brown (lower image).



Ph. 5-6 Comparison of dip-dyed acrylic fibres.
Bright field mode; side-by-side view; total magnification 400x; (K | Q).

Apart from the fibre colour transitions, other important characteristics are the diameter, the cross-sectional shape and the delustrant size and distribution.

5.3 CASEWORK EXAMPLES

In this section, five casework examples are discussed. Only bright field characteristics and fluorescence colours are shown. The K and Q samples do not differ in polarization characteristics.

5.3.1 Case 1: Dark acrylic fibres

In one of our cases, dark acrylic fibre traces were found on a murder victim and on objects at the crime scene (Q). These were compared to fibre traces found in the backpack of a suspect (K). Photomicrographs of two different comparisons of this fibre type are presented in [\[Ph. 5-10\]](#).

In bright field mode, these bean-shaped acrylics have a diameter ranging from 15 to 25 μm . An inhomogeneous ring dyeing is present: the fibre has dark blue edges, while its centre is greenish. This fibre type is not delustrated. The fibres are heavily damaged: the lobes are split at the fibre extremities and inside the fibre.

Only very weak fluorescence is observed with near-UV excitation, and this was not documented. However, medium orange and red fluorescence are observed respectively with violet-blue and green excitation. Traces of blood are present in some zones of the questioned fibres. These surface contaminants can affect the fluorescence.

5.3.3 Case 3: Reddish cotton fibres

Red-orange cotton fibres were found as possible target fibres on the body of a murdered woman. At the residence of a suspect, an orange T-shirt was seized. A comparison of this cotton type was performed in a side-by-side view. The comparison in [Ph. 5-13] mainly illustrates the corresponding fibre colour in bright field mode and the corresponding fluorescence with the three fluorescence cubes.



Ph. 5-13 Comparison of orange-red cotton fibres.

The control fibres, coming from an orange top of the suspect (K), were compared against a fibre trace found on the victim (Q). Bright field and fluorescence mode; side-by-side view; total magnification 400x; (K | Q).

5.4 QUALITY ASPECTS

5.4.1 Training

At first sight, comparison microscopy is similar to the basic microscope methods already discussed in the previous chapters. However, certain practical aspects are somewhat harder to realize and require proper training. A checklist such as the one in [Tab. 5-1] can be used in training fibre examiners. It can help the trainer to cover all practical aspects of comparison microscopy and follow the trainee's progression.

Tab. 5-1 Checklist for training in comparison microscopy.

<p>Practical beginner skills</p>	<ul style="list-style-type: none"> <input type="checkbox"/> Setting up Köhler illumination <input type="checkbox"/> Verifying microscope settings <input type="checkbox"/> Obtaining proper background colour compensation <input type="checkbox"/> Comparing samples <input type="checkbox"/> Using photomicrography 	<p>any sample " " " " " " a set of K and Q samples for documenting characteristics in <i>side-by-side</i> and <i>butterfly view</i></p>
<p>Advanced skills (1)</p>	<ul style="list-style-type: none"> <input type="checkbox"/> Using photomicrography 	<p>a set of K and Q samples for documenting characteristics in <i>continuous view</i></p>
<p>Advanced skills (2)</p>	<ul style="list-style-type: none"> <input type="checkbox"/> Description of fibre characteristics in bright field mode <input type="checkbox"/> Description of polarization characteristics (dichroism, interference colours) <input type="checkbox"/> Description of fluorescence 	<p>a set of K and Q samples, including</p> <ul style="list-style-type: none"> • man-made fibres of different generic classes with interesting characteristics; • vegetable fibres that possess fluorescence or dichroism; • animal fibres that possess fluorescence or dichroism

5.4.2 Quality assurance

Correctly using a comparison microscope is not that easy. Adequate training should be provided to any operator. Here are a few obvious but essential issues that might cause difficulties in the comparison process. These issues need attention during training:

- Köhler illumination should be set up for each microscope at the beginning of the session, and the field and aperture diaphragms of each microscope should be adjusted.
- Differences in background colour between the two microscopes should be adjusted.

CASE FILE: .../..	Fibre type: ...dark blue acrylics...	Sample # of known fibres (K): trace found in suspect's backpack	Sample # of questioned fibre (Q): trace found on the victim's body
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BRIGHT FIELD	Description	Similar? (Y/N)	Explain differences
Fibre colour	green centre, blue borders	Y	
Dye appearance (even, ring or zone dyeing)	ring dyeing	Y	
Cross-sectional shape	bean	Y	
Diameter range (min.- max.)	15 – 25 µm	Y	
Delustrants (presence, size, distribution)	none	Y	
Pigments (presence, size)	none	Y	
Fish eyes (presence, max. size)	no	Y	
Print or coating	no	Y	
Surface markings	no	Y	
Cross markings	no	Y	
Fibre extremities (mushroom, bulbous, brush-like shape, ...)	split lobes	Y	
Other observations:	Strongly damaged fibres, lobes are split at fibre extremities and in the middle of the fibre	Y	

FLUORESCENCE	Description	Similar? (Y/N)	Explain differences
Colour FLUO NUV	colour undefined	Y	
Intensity FLUO NUV	very weak	Y	
Colour FLUO VB	orange	Y	
Intensity FLUO VB	medium, varying intensities	N	presence of blood on Q
Colour FLUO GR	red	Y	
Intensity FLUO GR	medium, varying intensities	N	presence of blood on Q

POLARIZATION	Description	Similar? (Y/N)	Explain differences
Interference colours	Central colour @ ... µm thickness 1 st order grey, negative elongation	Y	
	Central colour @ ... µm thickness		
Dichroism	none weak medium strong	Y	
	Regular inverse dichroism		
	Intensity change Colour change		

CONCLUSION	Correspondence (K Q)?	Yes
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