CHAPTER 1

LOW POWER MICROSCOPY

1.1 INTRODUCTORY SECTION   The size of textile fibres

Microtraces are fragments of materials with a globular or fibrous form, having such small dimensions, the traces are difficult to observe with the naked eye. The size of globular particles is smaller than a needlepoint. For fibrous matter, at least one of the dimensions falls into the sub-millimetre range. The fibre length usually exceeds 1 mm, but the thickness varies from a few to several tens of micrometres. The micrometre (μm) or micron (μ) equals a thousandth of a millimetre (1 μm = 10^{-3} mm). A low power microscope – often called stereomicroscope – enables us to observe some fibre morphology at magnifications ranging from 5x up to 100x. The internal details of fibres are usually studied using a high power optical microscope, often at a total magnification of 400x. A scanning electron microscope (SEM) in backscatter mode can be used to obtain highly detailed images of the surface of these microtraces with high depth of field.

Textile fabrics are very diverse materials, and can these can produce several types of fibrous microtraces.

In general, man-made fibres are cut to a suitable length, especially if they are blended with natural fibres, as the latter are found often in short lengths. Cut man-made fibres of a few centimetres are called **staple fibres**. These short, individual fibres are most often found as transferred fibre traces.

Fibres of considerable length could be **filaments**. These none-cut fibres are used in the construction of fabric composed of multifilament yarns. This fabric should be worn frequently or heavily damaged for filaments to be shed.

Sometimes a few fibres (often less than 10) are found together in smaller **fibre tufts**. With frequent wear of a garment or use of textile materials, pilling can occur at the surface of the fabric due to friction. Large **fibre pills** are often very apparent traces.

In damaged garments or other textiles, **yarn fragments** can be transferred. In natural rope, entire fibre bundles can be lost, so-called technical fibres.

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The practical **field of view (FOV)** is the size in mm of the illuminated circular field seen at any specific magnification. The FOV is calculated by dividing the ocular field number by the magnifications of objective and zoom.

$$FOV = FN / (M_{Objective} \times M_{Zoom}) \quad \text{in mm}$$

Tab. 1-1 Properties of the Leica MZ12 stereomicroscope.

M_{Zoom}	M_{Total}	FOV (mm)	R (µm)	DOF (µm)
0.8x	8x	26.3	13.3	2140
1.0x	10x	21.0	10.8	1380
1.25x	12.5x	16.8	9.3	968
1.6x	16x	13.1	7.4	608
2x	20x	10.5	6.2	409
2.5x	25x	8.4	5.2	280
3.2x	32x	6.6	4.3	181
4x	40x	5.3	3.6	123
5x	50x	4.2	3.3	94
6.3x	63x	3.3	2.7	61
8x	80x	2.6	2.7	52
10x	100x	2.1	2.7	45

The **resolution R** is the distance at which two nearby points in the same observation plane can still be distinguished separately. The resolution is determined by the manufacturer using a calibrated target with line pairs of different thickness. The maximum number of line pairs per millimetre determines the resolution at a specific magnification. For instance, at a magnification of 8x, the number of line pairs observed in 1 mm is 75. This would correspond to a resolution of about 15 µm. At the highest magnification, the number of line pairs per mm equals 375, resulting in a resolution of about 3 µm. For comparison, the highest resolution that is obtained with high power microscopy is about 0,5 µm.

Instead of one focal plane, at each specific magnification, there is a certain range of heights around this theoretical focal plane at which the object is observed sharply. This range at which the object is "in focus" is called the **depth of field (DOF)**. In the example, the DOF obtained with low power microscopy lies approximately between 2 mm at the lowest magnification and about 0,05 mm or 50 µm at the highest magnifications. The DOF can be determined practically using a calibrated oblique target with lines at regular intervals.

The **working distance (WD)** is the space between the object and the objective lens. It depends on the focal distance of the objective and thus on its magnification. In this example, for a 1x objective, the working distance lies around 60 mm. Longer focal distances are more practical for mounting fibres under the stereomicroscope. These are obtained with a low magnification objective, a so-called long working distance (LWD) objective such as a 0,8x objective lens.

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[Fig. 1-6] allows for easy and systematical screening of a taping at the lowest magnifications.

Fig. 1-6 Screening of tapings using a search grid.
Example of an A4-sized search grid with rectangular cells and its application for searching fibres.

The search grid in this figure has 5 x 18 mm rectangular cells and is used for screening the vertical columns at low magnifications, with a field of view that is slightly larger than 18 mm. At the same time, this grid can also be used for screening the horizontal rows for higher magnifications up to 40x, for which the field of view slightly exceeds the 5 mm height of the cells. Some microscopists prefer a search grid with large square cells, e.g. an 18 x 18 mm grid, instead of rectangular cells because there are fewer lines that may distract during the scanning task.

In general, the whole surface of the taping is screened systematically under low magnification. Whenever a fibre trace is found that resembles the control sample, a higher magnification can be used to check details. Fibre traces that correspond with reference fibres - also known as control fibres - are dotted or encircled on the taping using a permanent colour marker.

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1.3.5 Counting fibres

If a population of fibres is found on a taping, it may be useful to obtain an estimate of the population size. The number of fibres can easily be counted using the stereomicroscope and a colony counter pen, such as the one in **[Fig. 1-7]**, often used in microbiology. A fast and systematical review of the taping and clicking each time a particular fibre type is indicated allows for precise counting. It is good practice to note the number of fibres counted on the corresponding taping.

1.4.2 Choice of mounting medium

The initial choice of the mounting medium is very important. First of all, the nature of the mounting medium will have an impact on microscopic observations. Most fibres have an isotropic refractive index of around 1.5. The internal fibre characteristics will be observed if one uses a mounting medium with a similar refractive index. If the refractive index is very different, surface characteristics will be observed.

A ray of light that enters a transparent medium with a different refractive index will undergo refraction, i.e. the light will deviate from the straight line. Three different situations can be distinguished, as illustrated in **[Fig. 1-10]**. Suppose the second medium has a higher refractive index than the first medium. The light will then be refracted towards the normal on the interface between the two media. However, if the second medium has a lower refractive index than the first medium, refraction occurs away from the normal.

In case the two media have equal (or very similar) refractive indices, the light will (almost) not refract at all at the interface. This situation occurs for transitions from glass to a mounting media for which both refractive indices are approximately 1.5.

The degree of refraction can be calculated according to the law of Snellius-Descartes :

$$\sin i_1 / \sin r_2 = n_2 / n_1$$

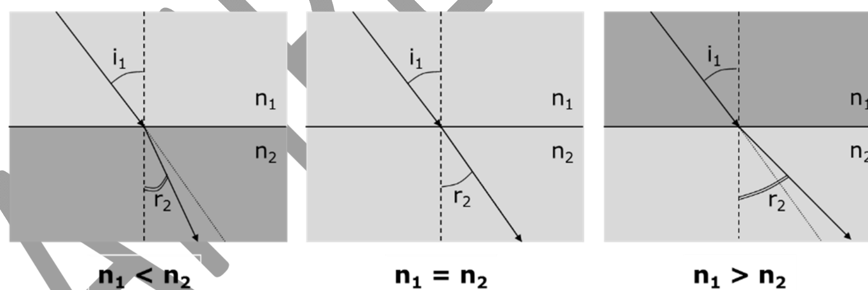

Fig. 1-10 Refraction of light.
Refraction occurs at the interface of two media with different refractive indices. No refraction occurs if the refractive indices of both media are similar.

The desired characteristics of a microscopy mounting medium destined for observation and long-time preservation are:

- it is a colourless, translucent material;
- it is an isotropic material, i.e. the medium has only one refractive index;
- its refractive index does not change considerably over time;
- it does not darken over time, although some slight yellowing may occur;
- it does not dissolve or attack fibres;

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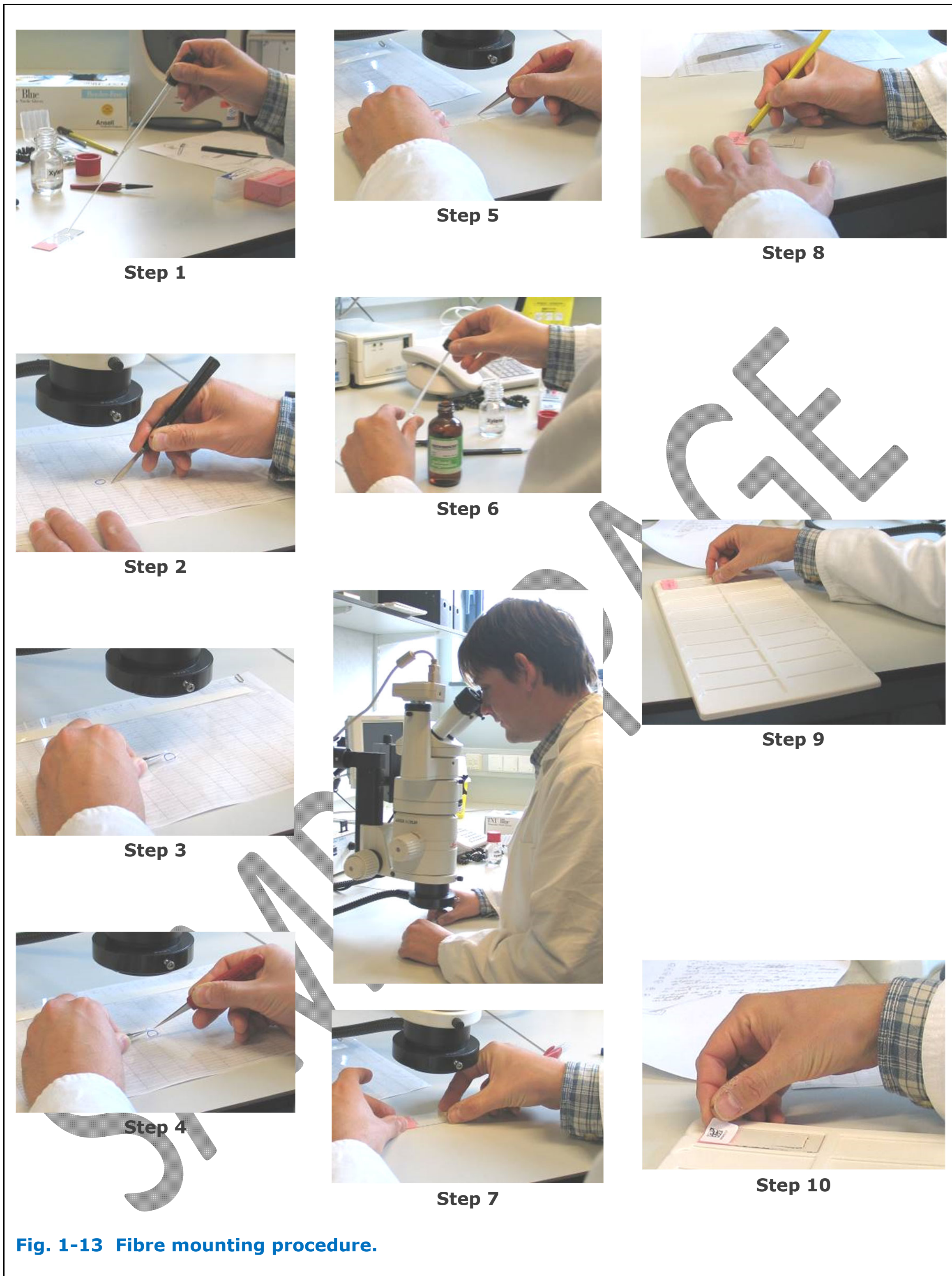

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Fig. 1-13 Fibre mounting procedure.

From a quality assurance perspective, it is essential to guarantee traceability. Therefore, it is good practice to individually mark each mounted fibre trace and reference (control) fibres with a unique code, allowing the evidence to be retraced to its origin. A drawing of mounted reference fibres and a mounted fibre trace is shown in **[Fig. 1-14]**.

1.5.2 A remark on photomicrographs

In the following sections, several fibre characteristics are illustrated with a series of fibre images taken at several magnifications. A scale is added at the lowest magnification, making use of a target grid. For didactical reasons, photomicrographs of the same fibre taken at 200x and 400x magnification are added too. This composition of fibre images will allow comparing the morphological details observed using both a stereomicroscope and a high power microscope. The fibres were not mounted using the procedure described in the previous section. Instead, a small piece of taping was cut out and fixed on a microscope slide with scotch tape. The fibres are enclosed in glue, and other microtraces or air bubbles may be present too. Because of the thick uneven glue layer and the presence of air bubbles, the quality of these images is lower than what will be shown in the next chapter on bright field microscopy. In general, the high magnification images taken with bright field microscopy in the transmitted light mode show internal fibre characteristics. In some of these images, surface characteristics such as the scales in animal hair are observed. This phenomenon is due to the difference in the refractive indices of trapped air and the fibre.
Comments on the observed characteristics are given in the captions of these photography plates. At this point, beginning fibre examiners may not fully understand some concepts, as further details are only explained in the following chapter. For them, it will be beneficial to return to the plates in this chapter after reading the chapter on bright field microscopy.

1.5.3 Fibre colour

The colour of fibres observed under different magnifications may vary. For instance, fibres appearing black at 10x magnification may be observed as purple (with a blueish shade) or as violet (with a reddish shade) at 80x magnification. As an example, the colour difference of the man-made fibres in **[Ph. 1-1]**, observed at 10x and 80x magnification is apparent.

Also, the perception of colour will be different depending on the type of illumination: reflected light or transmitted light. If the sample is illuminated from above, then its colour is determined by all wavelengths that are reflected at the surface of the object, i.e. the wavelengths that were not absorbed. On the contrary, if the sample is illuminated from below, its colour is determined by all wavelengths that are transmitted through the object.

Remark that the reflected light mode can always be used, while the transmitted light mode can only be applied for translucent objects.

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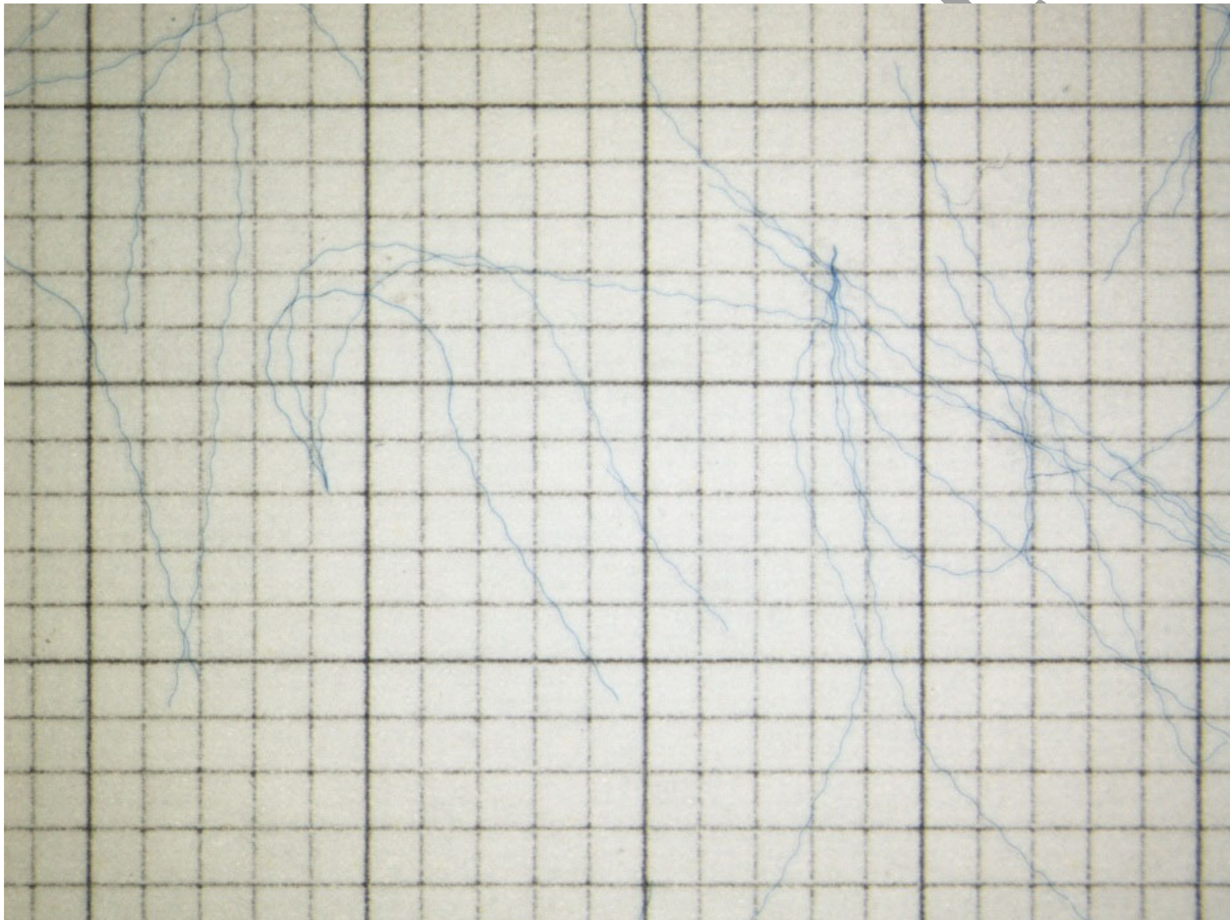
1.5.6 Waviness

The waviness or regular undulation of man-made filaments used in multifilament weaves is well visible at low magnifications. Both warp and weft yarns in this type of woven have different undulations. In woven fabric, the warp direction always has a higher yarn density than the weft direction. Therefore, the filaments coming from the weft direction will present more waves per unit of length. The waviness can easily be measured in waves/cm when placing the sample (microscopy slide or taping) on a millimetre grid, such as the one presented in **[Ph. 1-4]**.



Ph. 1-4 Regular undulations of fibre filaments.

1.5.7 Leather and microfibre tufts

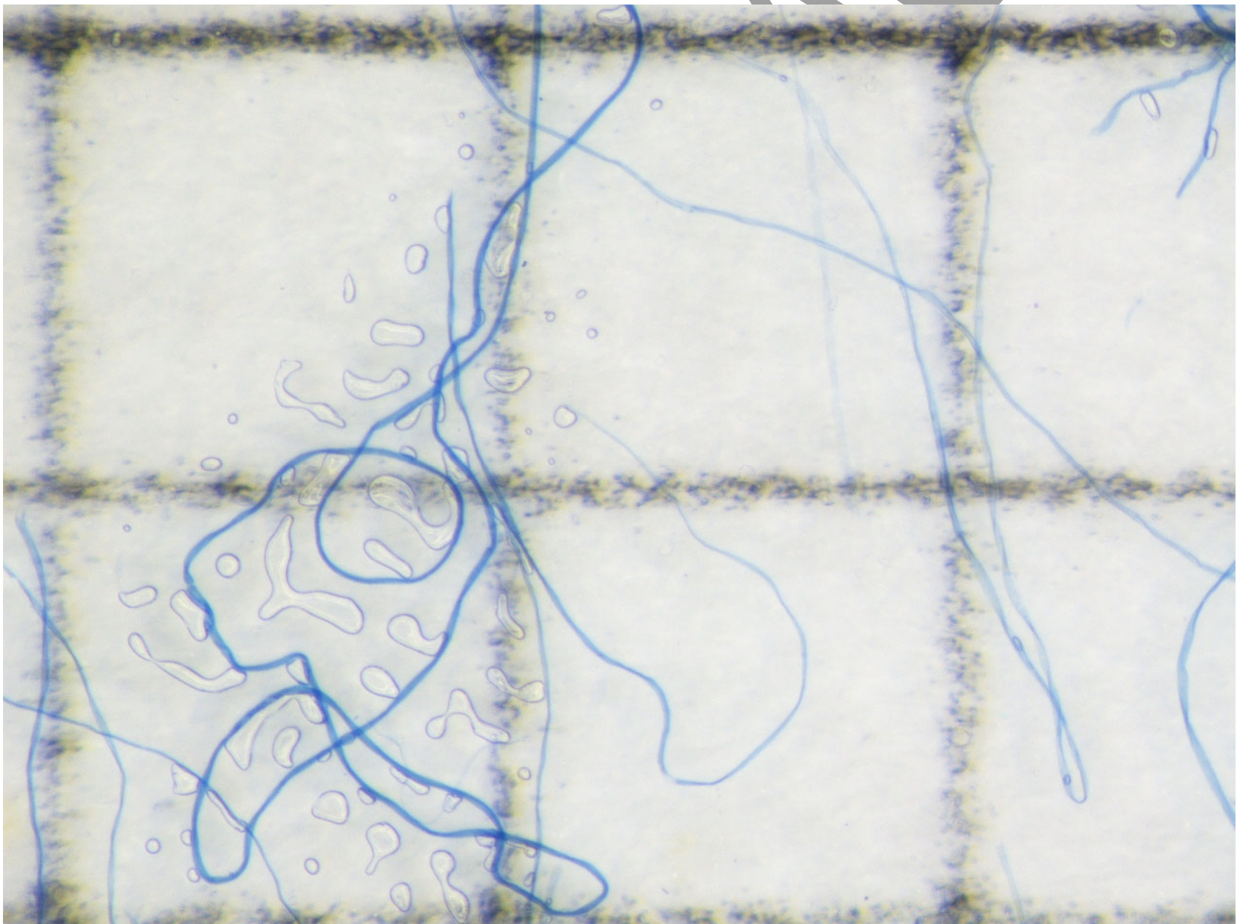
A leather particle is shown in **[Ph. 1-5]**. Leather is an easy fibre type to recognise as it is often transferred as thick but short tufts that are easily detected at low magnifications. The extremities of the particle are usually frayed due to the fibrillation of this natural material. At higher magnifications, the slightly wavy aspect of the aligned microfibrils can be distinguished, and dark bands may be visible where the fibrils change direction.

1.5.10 Cotton

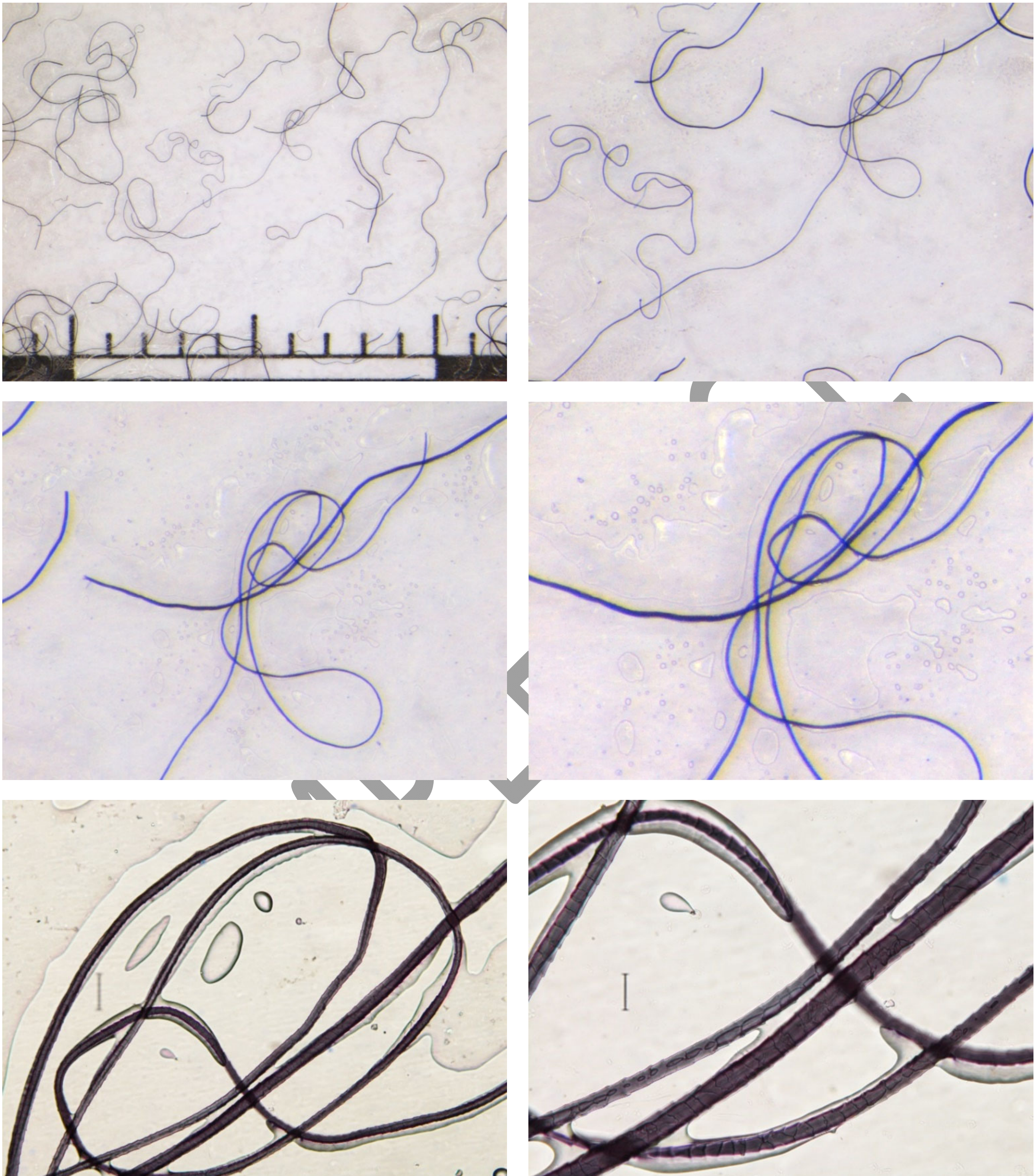
Cotton fibres have the typical morphology of a twisted ribbon. This natural fibre shows a high number of turns and directional changes. At low magnifications, these regularly occurring convolutions will be observed as a change in fibre diameter. The characteristic flow of cotton can be easily recognised using low power microscopy. Higher magnifications can confirm the presence of convolutions and the flattened morphology of the cotton fibre.

The example in **[Ph. 1-8]** shows a cotton fibre type on a millimetre grid at 50x magnification as an illustration of its typical flow, the variation in hue and diameter and the convolutions.

In **[Ph. 1-9]**, a frequently encountered cotton fibre type is shown. These are indigo dyed cotton fibres originating from denim, a fabric used for jeans.



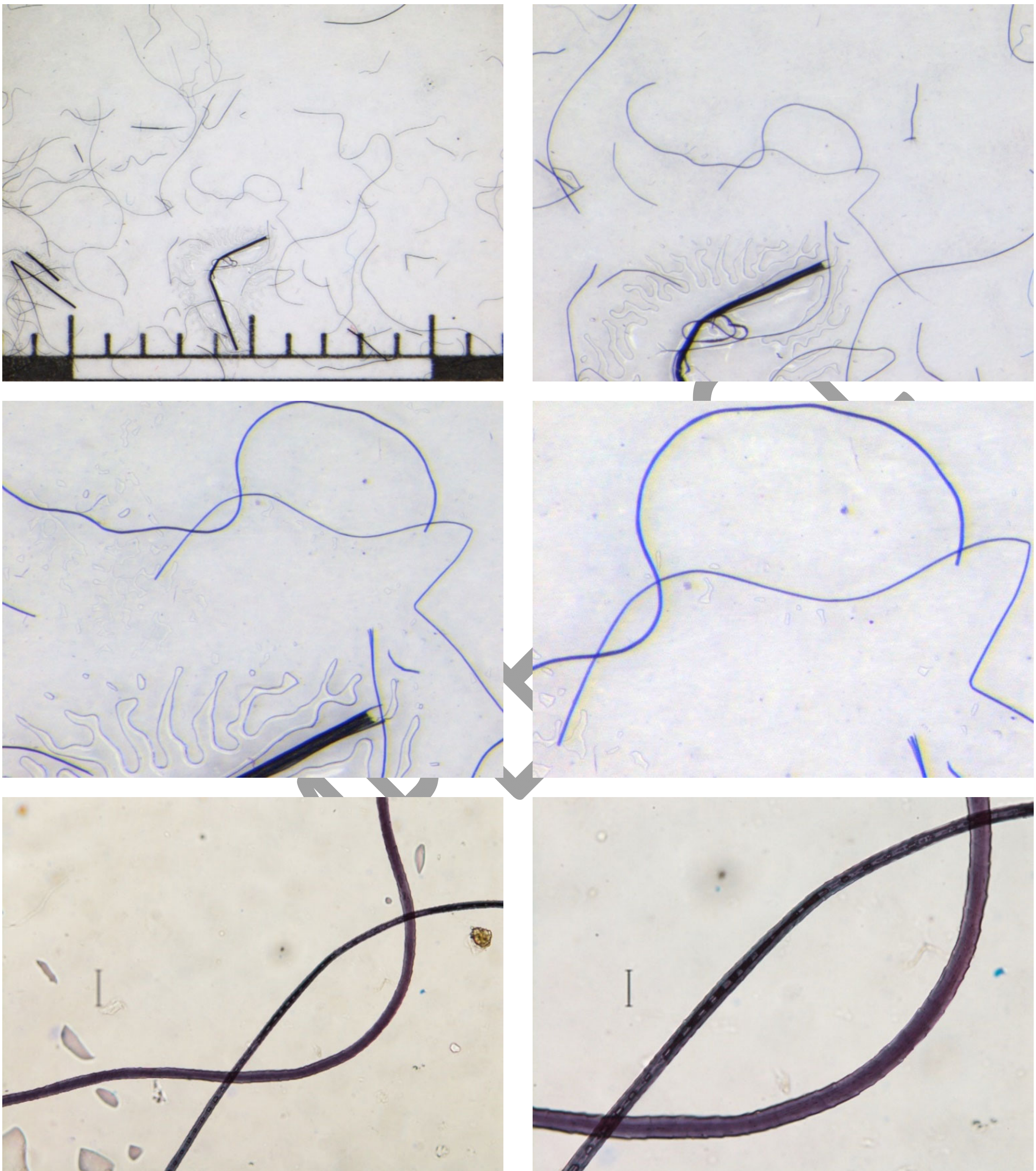
Ph. 1-8 Cotton morphology.



Ph. 1-12 Fine wool fibres, viewed at various magnifications.

Low power 10x	Low power 25x
Low power 50x	Low power 80x
Bright field 200x	Bright field 400x

Above and centre: The large range of fibre diameters is apparent, but the scales are hard to see at the highest magnifications with low power microscopy.
Below: In bright field microscopy at high magnifications, the scale pattern is observed due to air pockets at the fibre surface.



Ph. 1-17 Wool and angora, viewed at various magnifications.

Low power 10x	Low power 25x
Low power 50x	Low power 80x
Bright field 200x	Bright field 400x

Above and centre: The high crimp of wool and the smooth flow of angora is observed with low power microscopy. At the highest magnifications, protruding scales may just be observed.
Below: In bright field microscopy, no medulla structure is present in wool, while the uniserial ladder medulla of angora is observed.

1.6 TRAINING AND QUALITY ASSURANCE

1.6.1 Observation of colour

Colour vision depends on the functioning of three sets of colour sensing cones in the retina of the eye. These cone cell types contain different pigments that transmit signals to the brain whenever absorption of light takes place. The cone types have their highest absorption in the blue, green and yellow-green regions, often called blue (or short wavelength), green (or medium wavelength) and red (or long-wavelength) cones. When activated together, the receptors can detect colours in the whole wavelength range of the visible spectrum. People suffering from so-called colour blindness have some defects in one or more of the receptor cells. The most common forms are red-green colour blindness, blue-yellow and total colour blindness.

For an operator to successfully retrieve fibres, good colour vision is of extreme importance. Therefore a test for correct colour vision should be one of the criteria for selecting personnel. Colour blindness can be detected with the well-known Ishihara colour test. This test uses coloured circles that hide numbers. These are only visible if all cone cell types are correctly functioning.

1.6.2 Colour discrimination

Chromatic discrimination, i.e. the ability to differentiate between very similar hues, strongly depends on the observer. On average, it has been shown that the human eye has the highest discrimination in the green hue range and less discrimination for blue and red hues. However, the sensibility for a particular hue range can be high for one person and low for another. Therefore, the colour sensibility of each operator should be checked. This can be done with a Farnsworth-Munsell test. This test consists of four series of colour ranges. For each range, a number of coloured squares should be arranged in such a way that the hues gradually evolve from orange to yellow, from yellow to green, from green to blue and from blue to red. This test is designed to verify if there is decreased discrimination in one of these four colour ranges.

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