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Fundamental Principles of Fibre Fineness Measurement

Part 4

The Projection Microscope



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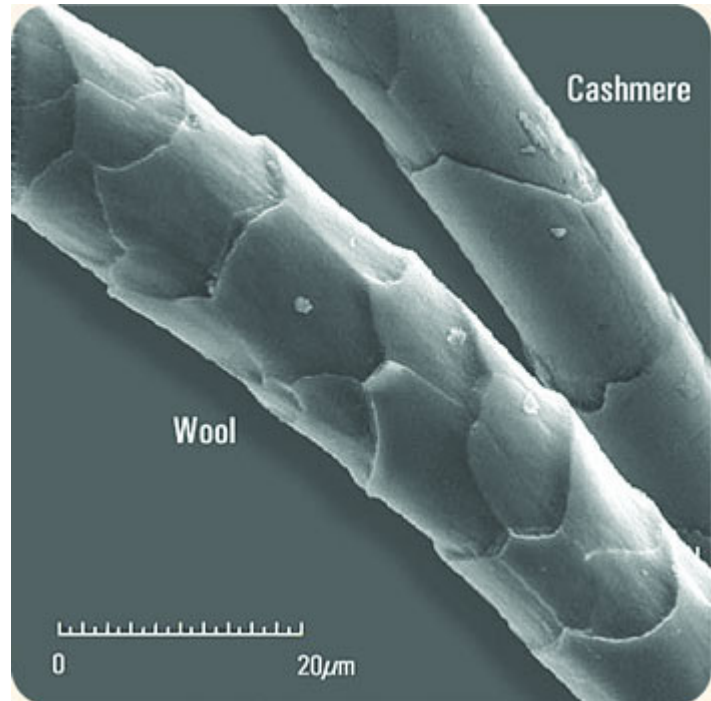
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THE PROJECTION MICROSCOPE

Early interest in the fineness of wool fibres was centred on wool top. The International Wool Textile Organisation (IWTO) initially defined fibre fineness in terms of the weight in milligrams of 10 metres of wool fibres at a regain of 18.25%. The method used (called the Gravimetric Method) relied upon weighing a defined number of wool fibres cut to a known length, and expressing the mean fineness in terms of the weight of a standard length at a standard regain.

This method, and consequently this definition, was subsequently found to have a number of limitations. In the period 1932 - 1954 an increasing emphasis was placed on the use of the Projection Microscope, which defines wool fibre fineness in terms of the mean width of the projected image of the fibre. The Projection Microscope was more precise than the Gravimetric Method and moreover it also provided information about the fineness distribution.

The American Society for Testing Materials (ASTM) produced a draft specification for the measurement of wool fibre fineness, based on the Projection Microscope, in 1950. The first IWTO Specification for the Projection Microscope was approved in 1954, following a series of international laboratory round trials in 1947 and 1948.



Direct Measurement of the width of magnified images of animal fibres remains the only primary method for determining fibre fineness, and the method against which all other methods must be calibrated

It is readily acknowledged that the Projection Microscope is of limited usefulness. Firstly it is a very slow and labour intensive technique. Secondly it is very imprecise when a single operator conducts measurements within one laboratory. High precision is only attained by using several laboratories and many operators.

Notwithstanding these difficulties, no alternative method exists for the direct measurement of the Mean Fibre Diameter of wool. *Consequently, the projection microscope remains the reference method against which all other instrumental methods, specifically the Airflow, SIROLAN-LASERSCAN and OFDA 100, must be calibrated*

Principle

There are two separate systems for estimating fibre fineness using optical microscopes:

- examination of the dimensions of cross-sections of fibres; and
- examination of the transverse dimensions of fibres.

Both systems enable estimation of the mean diameter and of the standard deviation in diameter, of the fibre population.



The first system requires obtaining thin sections across the transverse dimension of the fibres. In this instance, great care must be taken to avoid cutting at an angle to the longitudinal axis. Failure to do this will increase the cross-sectional area and increase the fineness estimate. The cross-sections can be viewed in either the transmission or the reflection mode. There are several techniques for mounting the cross-sections on a glass slide prior to measurement. The area of each fibre cross-section, magnified as a projected image, is measured using a planimeter or a similar device. Because of the difficulties inherent in sample preparation, there are no commercial standard test methods based on this technique.

The standard test methods developed by IWTO (IWT0-8) and ASTM (D2130-90) are based on measurements of the transverse dimensions of fibres. These require the distribution of a random sub-sample of the fibre assembly onto a glass slide, or the distribution of snippets, prepared from the fibre assembly using a microtome or a similar device, onto a glass slide. These fibres or snippets are distributed in a mounting medium, under a cover plate and generally viewed in the transmission mode, projected onto a screen. A number of techniques have been developed for estimating the physical dimensions. In general terms, these usually involve a graduated linear scale. The observer is required to classify the transverse dimension of each fibre into one of 40 or more class intervals, where each class interval is 2 microns. Thus a frequency histogram of the transverse dimensions of the fibres is developed. The sampling of the snippets is designed to obtain a length-proportioned sample and hence the measurement can be said to equate to the length-proportioned mean of the bulk.

The test methods are designed to ensure that measured snippets are selected at random, and that each snippet is measured only once at a single point located randomly along its length. Great care must also be taken to ensure that the snippets are in focus when being measured.

To minimise the effect of operator bias IWTO-8 requires the measurement to be conducted by at least two observers, each measuring 300 snippets.

The mean, \bar{d} , and the standard deviation, s , of the sample is calculated from the resultant histogram data.

$$\bar{d} = \frac{\sum_{i=1}^m n_i d_i}{\sum_{i=1}^m n_i} \quad 1$$

$$s = \sqrt{\frac{\sum_{i=1}^m n_i d_i^2 - \left[\left(\sum_{i=1}^m n_i d_i \right)^2 / \sum_{i=1}^m n_i \right]}{\sum_{i=1}^m n_i - 1}} \quad 2$$

- where n_i = the number of measurements assigned to the i^{th} class interval
 d_i = the diameter, in microns of the i^{th} class interval
 m = the number of class intervals
 i = an integer, 1,2,3,4..... m

A magnification factor of 500:1 is considered ideal.



Development

Early measurements of wool fibres were confined to measurements of single fibres. The first recorded use of a microscope was in 1777 when Daubenton measured the fibre thickness by comparison with lines drawn on a piece of quartz, which was also placed under the microscope. Adopting this technique Voightlaender (1815) and Winekler (1821) were the first to measure multiple fibres on the one slide. The fibres were mounted parallel to each other on a special frame, which was then placed under the microscope.

In 1860 Parry criticised Daubenton's method and was probably the first to actually measure the image of the fibre as shown by the microscope. About the same time Rohde introduced an eyepiece, equipped with micrometer.

In 1886 McMurtie described the Dollond Eriometer. This instrument, an adaption of Daubenton's technique, enjoyed wide usage in the early part of the 19th century and for some time it was considered the basis for comparison, the unit of measurement being the Dollond unit.

Doehner (1929) described an apparatus consisting of a microscope, with a mechanical stage. The stage was adapted to take a special cell consisting of a metal frame divided into three compartments, and carrying two glass plates between which the wool sample, previously cleaned by brushing over with ether, and mounted in thinned cedar oil, was distributed. A wooden box stood in front of the microscope and in a tight connection with it. The front of the box carried a matt viewing screen provided with a light protecting cap for daylight use, and a measuring disc or apparatus for photographing the projected image. If the matt screen was removed the image could be projected onto a wall for the benefit of a number of observers. The magnification for viewing on the matt screen was 60:1 and standards for comparison were provided by means of diapositives kept in a slide holder beside the apparatus.

These permitted the sample to be classified roughly. If more precise information was required, the thickness of single fibres was measured by means of a rotatable disc, calibrated in millimetres. In this case, the microscope was arranged to project an image magnified by 500:1 onto the graduated disc, and the width of the fibre at a given point was measured in millimetres. The calculated fibre width measurements were classified and the classified widths plotted as an abscissa, with the frequency of each classification as the ordinate. With practice, approximately 100 measurements could be made every 10 minutes with this apparatus. Barker (1931) designed a double optical system, which projected two images side by side, a test sample as well as a standard sample, for comparison.

Von Bergen (1935) commented that the old methods of measuring the thickness of the fibres through a microscope with a micrometer were too tedious and not sufficiently accurate. He too favoured projecting the image of the fibre onto a screen at high magnification and measuring its width, and developed a wedge ruler to simplify the measurement process. The width of the image was recorded on the wedge ruler in such a manner as to automatically sort the fibres according to their width.

At a lecture at Roubaix in 1935 Rasuch summarised the situation regarding fineness measurement, prior to the 1936 conference of IWTO, where Germany was proposing to discuss in full, methods for estimating the properties of wool. In his opinion the projection method, based on Doehner's Lanometer, was the most satisfactory.

Bernhad (1938) reported that the speed of the measurement was increased by using a plain frosted screen in the lanometer and by measuring the fibre thickness on this by using a transparent celluloid rule.

In 1938 IWTO decided that any satisfactory type of apparatus would be recognised for measuring fineness in cases of arbitration. However IWTO had adopted as one of its primary objectives the drawing up of standard methods, based on generally accepted procedures, which would serve to measure independently all the characteristics of wool entering into the assessment of quality.

Henning (1940) reported on progress by an IWTO technical committee in establishing a standard technique for measurement of wool fibre fineness based on the Projection Microscope.



Wollner, Tanner & Spiegel (1944) described a modification of Von Bergen's wedge method for estimating fibre thickness using projected images. The authors had developed a wedge rule base on a calibrated spiral. They also reported the preparation of very short snippets (approximately 75 micron in length) for measurement. This provided a compact single layer of fibre snippets on the microscope slide, minimising the need to refocus the instrument during measurement.

Anderson and Palmer (1947) provided evidence that measurement of mean width of fibre snippets by the Projection Microscope was sensitive to the snippet length. They examined snippet samples of two tops (both with a mean diameter in the range 32 - 38 micrometres), where the snippet length ranged from 50 to 1600 micrometres (0.05 mm to 1.6 mm). Results for very short snippets were significantly higher than for longer snippets. They attributed this to a tendency for very short snippets to come to rest on the slide, under the influence of gravity, with their major axis parallel to the slide. They concluded: **"it appears that for fibres of non-circular cross-section a section length of 300 microns is too small and it may be worth while adopting a minimum length of 800 microns, though this may be too small for some fibres. For merinos on the other hand, this effect is not likely to be so pronounced"**. WIRA (1955) published additional data, using tops of a similar diameter, confirming this effect and suggesting a minimum snippet length of 800 micrometres (0.8 mm) was required.

The effect of water absorption on the radial dimensions of wool fibres was also being extensively examined as it has implications for any wool fineness measurement system. The first published work was by Hirst (1922) who carried out microscopic measurements of a single wool fibre at a number of different regains and demonstrated the increase in the dimensional characteristics of the fibre as the regain increased. King (1926) conducted some quantitative experiments and was able to calculate the radial swelling of the fibres for a range of increasing regains. Warburton (1947) demonstrated that increasing the regain from 0% to approximately 32% increased the radial dimensions by approximately 17%.

Cassie (1945) reported a study of the absorption isotherms of water into wool fibres. He explained an observed hysteresis effect in the adsorption-desorption process in terms of a mechanical hysteresis of the fibres. The implication of this work is that measured fineness of wool fibres is influenced by the mode of equilibration with water. Conditioning from the dry side produces a different effect to conditioning from the wet side.

Semple (1947) considered the interaction of the absorption of moisture and the buffering capacity of the mounting medium on Projection Microscope measurements. He suggested that there was merit in heating the mounting medium rather than attempting to maintain control of either the water content in the medium or the conditioning of the wool.

Anderson & Palmer (1948, 1951) considered this issue in some detail. They concluded that there were two ways of mounting wool fibres for Projection Microscope measurements that are both satisfactory, in principle, for diameter measurements:

- condition the wool and mount in a medium such as cedar wood oil that has a low water buffering capacity; or
- do not condition the wool and mount the fibres in a medium such as glycerine or water that has a high buffering capacity.

Mediums of intermediate buffering capacity should be avoided. If wool is mounted in mediums of high buffering capacity, then the final regain of the fibre will be determined by the medium, and not at all by the initial regain of the wool. If wool is mounted in a medium of low buffering capacity, such as cedar wood oil, then the final regain of the fibre will be the same as when it was mounted, irrespective of any moisture content the oil might have. Anderson & Palmer also suggested that the refractive index of the medium was not a critical factor in determining the fibre diameter. In spite of this they suggested that some mediums might give less observer error than others owing perhaps, to easier focussing.



In 1947 a round trial was conducted, to evaluate a Projection Microscope developed by the Wool Industry Research Association (WIRA) in the UK. The trial used 5 tops ranging from 21 to 37 micron and involved laboratories in UK (WIRA), Belgium, Canada, USA and Italy. No special effort was made to standardise the procedures to be used in the participating laboratories.

This particular trial indicated that differences between the laboratories were not significant, and that most of the variation in the measurements seemed to be due to between operator variances within laboratories. The standard deviation of the results was 0.53 microns, or a precision of ± 1.06 .

A more extensive trial, involving 15 laboratories was organised in 1948 and the results reported by Palmer (1948). The objective of this was to test the reproducibility of the measurements when all laboratories strictly followed the same procedure.

- It is worth noting the special points in the procedure that were adopted in the 1948 trial.
- A standard snippet length of 800 microns (0.8 mm) was adopted.
- The fibre pieces on the slide were brought into equilibrium with an atmosphere of 65% R.H. and mounted in a medium of low buffering power such as cedar oil.
- Selection rules were designed to ensure that the observer measured the fibres at a place absolutely independent of any observer choice.

The precision of the mean diameter measurement was ± 1.26 , a little higher than the 1947 trials. Palmer observed: **"Neither of these could be regarded as satisfactory, because an error the size of the smaller means that two laboratories will differ by 1 micron or more about 1 time in six"**. However Palmer also observed that for the diameter measurement 6 of the 15 laboratories were "out of control" in that their deviation from the others was statistically significant.

The 1947 and 1948 trials were major milestones in the development of Projection Microscope standards. Shortly after the completion of these trials a tentative ASTM specification for determination of wool fibre fineness of raw wool, top and yarn by the Projection Microscope was published. By 1954 the Projection Microscope method was progressed to a standard test method by IWTO. However, while these standards have continually improved, little substantive development to the instrument, apart from improved optics, has occurred.

The human factor has always been one source of variation in the method. A comprehensive study of operator bias and its day-to-day variation was reported by Kritzing et al (1964).

Precision

The precision of the Projection Microscope for the measurement of fineness of wool top and greasy wool is defined by IWTO-8 (IWTO, 1989). The standard states that **"...in the absence of more definitive data, the estimates of the variance components calculated by Andrews and David (1978) are the best available"**. These data are shown in the Table on the next page.





Variance components of Mean Fibre Diameter by Projection Microscope

Component of Variance	Symbol	Value (for raw wool)
Between Bales	s_t^2	0.125 (μm^2)
Between cores	s_c^2	0.083 (Australian Wools)
Between laboratories	s_l^2	0.082
Between sub-samples	s_s^2	0.024 (for $d = 22 \mu\text{m}$)
Between specimens	s_k^2	0.011
Between operators/slides	s_o^2	0.058
Between fibre snippets	s_f^2	25 (for $d = 22 \mu\text{m}$)

Using these data an estimate of the variance of the method for a 22 micron lot of raw wool can be made from the formula:

$$S^2(d) = \frac{s_c^2}{n} + s_l^2 + \frac{s_s^2 \left(1 - \frac{q}{5}\right)}{q} + \frac{s_k^2}{k} + \frac{s_o^2}{kj} + \frac{s_f^2}{ijk} \quad 3$$

- where
- n = total number of cores taken from the lot (each bale being equally cored)
 - q = subsamples taken from the total sample of n cores after blending
 - k = test specimens taken
 - j = slides prepared from each test specimen
 - i = fibre snippets measured from each slide by one of 2 operators

The 95% confidence limits or precision are then given by:

$$95\% CL = 1.96 \sqrt{S^2(d)} \quad 4$$

For raw wool, where the samples have been obtained by core sampling, the confidence levels are therefore ± 0.87 for a 22-micron lot. IWTO-8 provides additional equations to allow estimates of the precision for a range of Mean Fibre Diameters. A similar calculation can be done for estimating the precision when measuring wool top (sliver).

The between laboratories component of variance is the largest single component, accounting for over one third of the total. If 400 or more snippets are measured, the effect on the between snippets components on the variance of the mean is outweighed by the combined contributions of the other components, particularly the between laboratories component. Beyond a certain point it is unproductive to attempt to reduce further the over-all variance of the mean by increasing the number of snippets. It follows therefore that in order to improve the precision of the Projection Microscope estimate of mean diameter it is necessary to replicate the testing in more than one laboratory.

Lunney (1980) considered the effect of random errors of observation on estimates of mean diameter. The projection method requires the observer to categorise individual fibre measurement into class intervals of 2 micron. Random errors of observation result in measurements being placed in an adjacent class interval.



Lunney simulated this by perturbing the distribution. He moved one quarter of the elements in each class interval to the interval immediately below, and one quarter to the interval immediately above. This simulation showed that resulting increase in between-fibre variance may be neglected. Lunney concluded that random errors of observation of individual fibres do not contribute significantly to the variance of the method.

Sheppard (1898) suggested that variances of continuous distributions calculated from frequencies assigned to discrete classes of identical class interval, over-estimated the variance (and hence the standard deviation) of the distribution. Sheppard suggested that a quantity $h^2/12$, should be subtracted from the variance, where 'h' is the class interval, to remove this bias. David (1992) used computer simulation to determine whether this correction should be applied to Projection Microscope measurements on wool. He concluded that the bias does exist, but that it is variable, apparently at random. Panov (1995) reviewed David's paper, pointing out that the problem in using the Projection Microscope was the range in error in measuring transverse dimensions of individual fibres. Sheppard's correction of 0.333 is negligibly less than the class interval (2 microns), and less than the lower detection limit (LDL) of the Projection Microscope, estimated to be approximately 1 micron. Consequently the error in measurement is greater than the bias introduced by using grouped data to determine the mean and the variance.

Commercial Importance

Although the Projection Microscope is now rarely used as a basis for the commercial trading of wool, its importance to the industry, commercially and technically, cannot be overstated. It remains the only standard method that can provide both a mean transverse dimension and a standard deviation in that dimension, by direct measurement. It is the only such method in current usage, for which an international standard exists, and is therefore the primary reference method for the industry. It is the basis for calibration of all alternative commercial instruments.

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