Heritage Science

REVIEW

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Identifying plant fibres in cultural heritage with optical and electron microscopy: how to present results and avoid pitfalls

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Abstract

Identification of archaeological and historical textile fibres is important because it gives insight into resource management in former times. The arrival of new tools such as table-top scanning electron microscopes, have led to an increased interest in the topic. Unfortunately, there have been cases where a lack of documentation regarding instrument settings and selection criteria has led to questionable conclusions being drawn. Optical and scanning electron microscopy are powerful techniques, but they must be used correctly and with proper knowledge of their limitations. Furthermore, ancient fibre material is often difficult to examine due to issues such as sample degradation, mineralization and the scarcity of material, which means that conclusions based on a statistical analysis of a large number of fibres are essentially not possible. In a cultural heritage context, it is therefore essential to distinguish between characteristic features, by which we mean features that are often, but not always present in a particular species and distinguishing features, which are always present in a particular species and can therefore be used for identification even if only a small amount of sample material can be examined. We argue that the community will have to accept that, quite often, a secure identification is not possible and that absolute statements such as: "This textile is made of flax" will often have to be replaced by relative statements such as "The material is likely to be flax". In this paper, we address these issues as follows: first, we present a fibre identification diagram which can be used, with some limitations, to distinguish between flax, hemp, nettle, jute, hops, and cotton using optical and electron microscopy. We then move on to highlight some of the typical pitfalls of using optical and electron microscopy for fibre identification. Finally, we present measurement documentation tables for optical and electron microscopy images, which we suggest should always be included in publications. Material scarcity means that the amount of material used for investigations should be kept at an absolute minimum. It is thus crucial that results are published with proper documentation so that measurements do not need to be repeated (more material is used) in future studies. It is our hope that the measurement documentation tables will be adapted by the community and used in future publications in the field. The paper finishes with a demonstration example, presenting a fibre analysis of Viking Age textile fragments from the 10th Century with documentation tables.

Keywords Identification, Microscopy, Flax, Hemp, Nettle, Jute, Hops, Cotton, Cultural heritage

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Introduction

Textiles have been crucial for society throughout history. In many areas of the world, they are as critical for survival as food and water, and they have always played an essential role in the demonstration of gender, age, social-, political- and economic status, as well as occupation, religion, and ethnicity ([1], p.51–54, [2]). It has

been suggested that textile crafts date earlier than metallurgy and even pottery [3]. A recent find of a Neanderthal tree bast string from Abri du Maras in France dates back to around 50,000 years ago, suggesting that the beginnings of textile crafts are even much earlier than hitherto believed, 20,000 years ago [4, 5]. The first major revolution in human societies: the transition from a hunter-gatherers to an agricultural society [6, 7], was naturally not driven by textile production alone, but the transition from the use of wild natural resources such as tree bast, nettle and fur to agricultural products such as flax, hemp and wool had a significant impact on ancient societies [8–11]. The importance of textiles is also highlighted by the fact that one of the most important events in modern history: the Industrial Revolution, was driven by the textile industry through innovations of mechanical spinning- and weaving machines [12].

Information about what kind of materials have been used to produce archaeological and historical textiles is very important because it provides knowledge about the infrastructure and resource management in the societies where the objects were made and used, as pointed out already by one of the early textile historians Agnes Geijer [13]. This insight, combined with the occurrence of new and/or more easily accessible identification instruments (such as tabletop scanning electron microscopy), has led to a massive increase in the work on the material analysis of textile heritage objects. This is a very welcome development, but unfortunately, some confusion regarding what features can really be used for the identification of textile fibre species has given rise to some misleading conclusions in recent and former times. Part of the challenge is that the material resources of former times differ from the modern ones. In the past, a larger variety of fibres were used than the limited number of commercial fibres used nowadays. The identification diagrams, derived mainly from industry and forensic science, depend on relevant species. The identification diagrams cannot be correct if relevant species used in the past are not included [14].

The aim of this paper is threefold: firstly, we show, based on the state of the art in the field, to what extent it is possible to distinguish between the fibres flax, hemp, nettle, hops and cotton using optical and scanning electron microscopy. We introduce the concepts of characteristic features and distinguishing features (see the next section for exact definitions) to clarify what can be done and what not and present an identification diagram used for plant fibre identification. The study is useful in a context of European cultural heritage mainly. Techniques such as synchrotron X-ray microdiffraction (μXRD), Fourier Transform Infrared Spectroscopy (FTIR), Raman Spectroscopy, Wide-angle X-ray scattering (WAXS), micro-CT, and analysis of ancient deoxyribonucleic acid (aDNA) have also been applied. However, so far, none of these techniques has provided a breakthrough in identification work for plant fibres. aDNA analysis, which would seem the most promising, has so far not been successful because usually, the DNA is destroyed by degradation. For instance, the retting process of plant bast fibres makes this technique insufficient even for modern fibres [15–25].

For well-preserved, individual plant bast fibres, the best possible identification can currently be done by using a polarised light microscope. There have been several successful studies on plant fibre identification in European cultural heritage textiles [26–29]. However, none of them includes the new concept of distinguishing- and characteristic features that is explained in this paper. Scanning electron microscopes can provide good images of surface features and is justified to be used when the fibres are mineralised and/or impossible to separate, but the identification that can be done based on scanning electron microscopy alone is limited.

Secondly, we show some examples of pitfalls, demonstrating how the incorrect use of optical and scanning electron microscopes can cause misleading conclusions.

Finally, we present measurement documentation tables for optical and scanning electron microscopy investigations. We recommend that results should always be presented together with such tables (which can typically be included in a Additional file 1a section). This is an important ethical issue. Optical and electron microscopy investigations are destructive techniques, fibre material needs to be removed from the original object. Proper documentation is of the utmost importance because it ensures that the measurements can be used for other future investigations without the need to sacrifice more material. Moreover, publishing the full set of technical data regarding the experimental measurement conditions opens the possibility of re-analysing an image in the future from a different point of view (looking for different information) than what was initially considered.

We finish the paper with a demonstration example: an investigation of Viking Age textile fragments following the procedures we present here.

Characteristic features versus distinguishing features for textile plant fibres

Well-preserved textile fibres can usually be identified as animal or plant fibres in an optical or electron microscope because the two groups differ from each other to a large extent. For instance: animal hairs usually have scales, very well visible in a scanning electron microscope (Fig. 1a), silks are smooth and glossy, and plant fibres have either dislocations (nodes) and

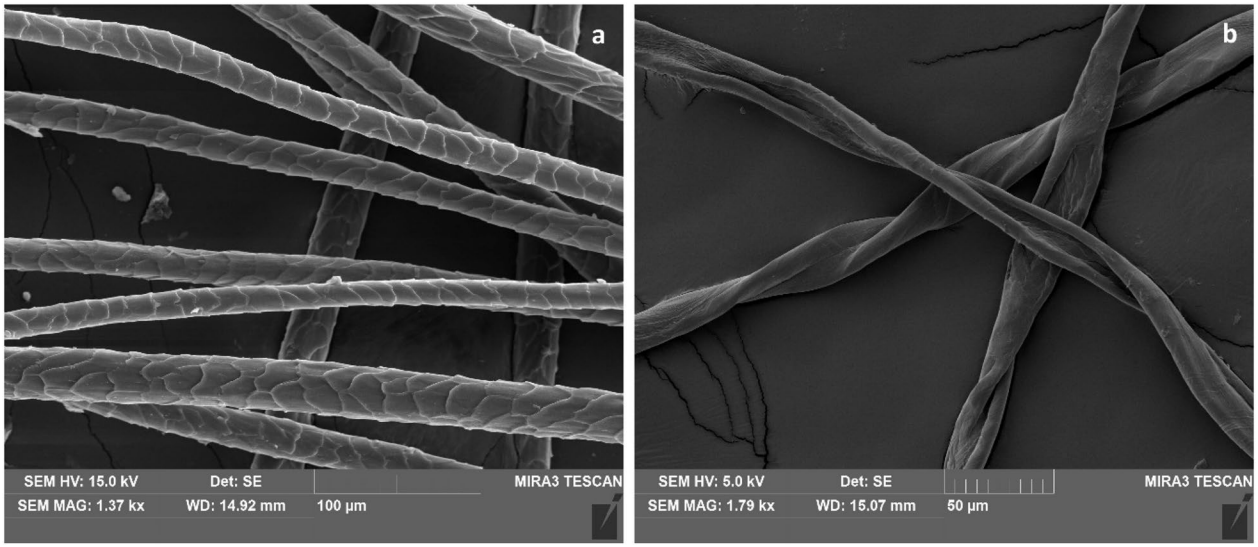

Fig. 1 Scanning Electron Microscopy—Secondary (SEM-SE) micrograph of sheep wool (on the left); SEM-SE micrograph of cotton fibres with clear convolutions (on the right), © Lukesova

cross-markings or typical convolutions (Fig. 1b). However, determining the exact animal or plant species is much more difficult. Historical processing methods can differ from modern ones which may impact the fibre's appearance and preservation of identification features. The material of historical and archaeological objects is often degraded, which may cause identification features to be changed or missing. This requires specific knowledge related to sampling and interpreting of results and limits the methods that can be used. For instance, carbonization prevents the use of transmission light microscopy [14].

Natural plant fibres used for textile production can be divided into three main groups. depending on what part of a plant they appear: (i) Herbaceous and arboreal bast fibres (e.g. Flax, Hemp, Nettle, Jute, Hops, Lime, Willow), (ii) Seed/fruit hairs (Cotton, Kapok, Fireweed, Cottongrass) and iii) Leave fibres (e.g. Sisal, Cordyline, New Zealand flax). An overview of some selected fibres and fibrous materials used for textiles and heritage objects in the past has been introduced by Lukesova ([14], p.111).

Many plant fibres are pretty similar in appearance and structure (which makes species identification so difficult): A plant fibre consists of a central empty space (lumen) surrounded by a cell wall which divides into (a) primary- and (b) secondary cell-wall which again is divided into sub-layers as well as (c) middle lamella or intercellular layer, which fills/divides the space between two neighbouring cells (Fig. 2). Some authors refer to an additional tertiary cell wall that is the innermost part of a cell [30–32].

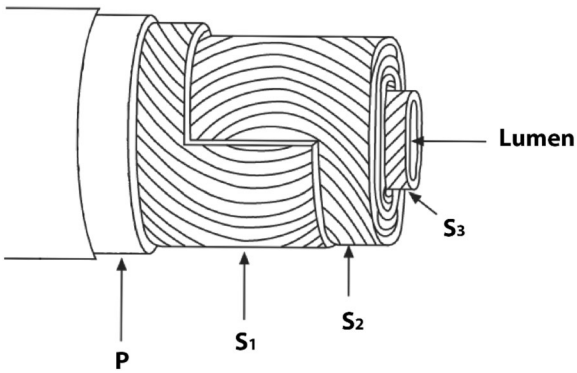

Fig. 2 An illustration of cell-wall structure of the cotton fibre, after Morton and Hearle [32]

Some species have remains of so-called protoplasm inside the lumen that can have a thin ribbon-like appearance, e.g., ramie and flax ([33], p.124). The fibre cells elongate during plant maturation. Thus, immature fibres are shorter than mature ones. The length of a fibre is one of several features related to the quality of a material.

Before we continue, we introduce two very important concepts for fibre identification:

Firstly, we introduce the concept of characteristic features, which, as the name says, are typical for a species: A characteristic feature for a species occurs often but not always (i.e. a narrow lumen for flax), and the feature can occasionally occur in other species. This means that such feature can only be used for identification purposes when working with high number of samples (at least 100

samples following the ISO standard 20706-1:2019). This is often not possible due to ethical reasons when sampling heritage material. Furthermore, characteristic features can be used, at the most, to conclude that fibre is likely to be of a specific species. For instance, a characteristic feature of nettle (*Urtica dioica*) are flattened areas of a fibre (Fig. 3a, on the left) that occur sometimes, but not always and may also occur in other species. Typically, fibres with flattened lumen showing rounded edges (Fig. 3b, on the right) tend to create flexions like a flattened tube [36].

Secondly, we introduce the concept of distinguishing feature, which is a feature that is always present in a fibre of a particular species. Note that this does not mean that the fibre can be identified with certainty; more species may share the same feature, but if it is possible to narrow down the group of species, then in some cases distinguishing features can be used for secure identification, i.e. given the choice of nettle, hemp and flax, Z-twist (see section Polarized light microscopy) shows with certainty that the fibre is hemp. Sometimes, a combination of characteristic and distinguishing features can give specific identification. Continuing with the example above, the distinguishing feature S-twist combined with the characteristic feature: the presence of oxalate crystals in the surrounding (associating) tissue, identifies the fibre with certainty as nettle. However, the characteristic feature of nettle—calcium oxalate crystals—is challenging to find especially in archaeological fibres. A fibre showing an S-twist by the modified Herzog test without crystals should be identified as possibly flax. Moreover, an absence of flexions—a much more frequent characteristic feature typical for nettle, is an indication that a fibre is probably flax.

We note that so far, none of the alternative examination techniques mentioned in the introduction have been able to contribute to new distinguishing features. The following morphological features have been used over time for identification: dislocations/nodes and cross-markings, fibre length, cross-section diameter, lumen. Diameter, cross-section shape and lumen shape and fibre cell ends [33–38]. All of them have been refuted as distinguishing features [15, 33, 38–41]. It was suggested to use them only as indications if they are used without combining them with distinguishing features. Studying the earlier microscopists, one can notice there is a clear shift in the timeline: the first authors such as von Höhnel and Herzog [41–43] performed many measurements and came with rather modest claims. The second-generation elaborated it and drew conclusions Luniak and Koch [33, 44, 45]. The generations coming after often reused, what has been written in a rather simplified way, and claimed characteristic features to be distinguishing features as Gale & Cutler, and Carr et al. [34], p.412, [35] p.65; [79–83]. Mistaking characteristic features for distinguishing features is the most common error in publications on plant fibre identification in heritage objects.

All the morphological features in Table 1 can be identified using a standard transmitted light microscope (TLM). Since this instrument relies on the transmission of light through the object, it cannot be used to examine mineralised fibres and/or fibres that cannot be separated as individual fibres.

Micro-chemical tests using cuoxam has been used in addition to morphological features in to define a specific swelling behaviour of species such as flax, hemp, nettle, jute and hops [33], p.80, [36], p.281–282, [47, 49], p.58–62. Cuoxam is tetraamminediaquacopper dihydroxide

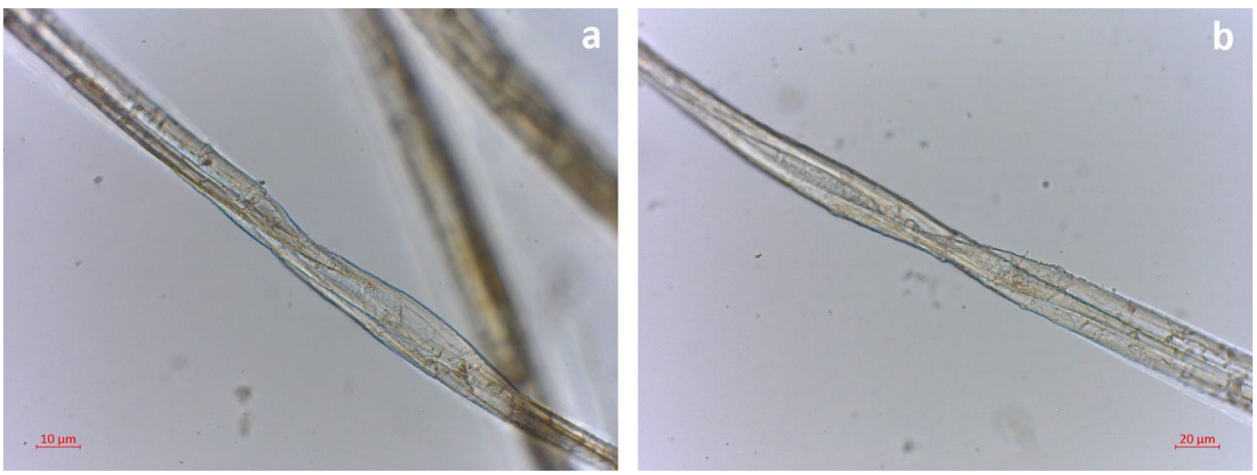

Fig. 3 **a** Nettle fibre (*Urtica dioica*) showing flattened area, transmitted white light microscopy (on the left); **b** nettle fibre (*Urtica dioica*) showing clear flexion, transmitted white light microscopy (on the right), © Lukesova

Table 1 Evaluation of plant fibre morphological features for identification of species; following publications are cited [15, 33, 34, 36–38, 40, 41, 46–48]

Morphological feature	Evaluated as diagnostic	Use with caution or as indication	Refuted
Fibre cell length	Gale and Cutler 2000, 412 Carr et al. 2008,79–83	Luniak 1953, 121 Wülfert 1999, 280 Petraco and Kubik 2004, 89	Catling and Grayson 1982, 78
Fibre cell ends	Gale and Cutler 2000, 412		Herzog 1955, 319 Catling and Grayson 1982, 2
Dislocations and cross-markings	Gale and Cutler 2000, 412	Wülfert 1999, 280 Petraco and Kubik 2004, 89	Luniak 1953, 122 Catling and Grayson 1982, 2 Bergfjord and Holst 2010, 957
Cross-section diameter	Carr et al. 2008, 79–83	Luniak 1953, 121 Wülfert 1999, 280 Petraco and Kubik 2004, 89	Catling and Grayson 1982, 78 Bergfjord and Holst 2010, 1194
Lumen diameter (ev. the thickness of cell wall)	Catling and Grayson 1982, 2 Gale and Cutler 2000, 17	Luniak 1953, 121 Petraco and Kubik 2004, 89	Bergfjord and Holst 2010, 1194
Cross-section shape	Luniak 1953, 122 Catling and Grayson 1982, 4 Gale and Cutler 2000, 412 Carr et al. 2008, 79–83	Wülfert 1999, 280 Petraco and Kubik 2004, 89	Luniak 1955, 319 Lukesova and Holst 2021, 224
Lumen shape	Luniak 1953, 122 Carr et al. 2008, 79–83	Wülfert 1999, 280	Lukesova and Holst 2021, 224
Cell structure/ Convolutions/flexions	Gale and Cutler 2000, 214 Carr et al. 2008, 79–83 Lukesova et al. 2019, 501	Wülfert 1999, 280 Petraco and Kubik 2004, 89	
Crystals/ Crystal shapes	Catling and Grayson 1982, 3 Luniak 1953, 125 Gale and Cutler 2000, 412 Petraco and Kubik 2004, 107 Carr et al. 2008, 79–83 Bergfjord and Holst 2010, 1193 Marková 2019, 26		
Adhering tissues as spiral elements, vessels and parenchyma cells, epidermal cells	Herzog 1955, 253 Catling and Grayson 1982, 3 Luniak 1953, 125 Gale and Cutler 2000, 412		

$[Cu(NH_3)_4(H_2O)_2](OH)_2$. The test has been used as a standard procedure for plant fibre identification.

Polarized light microscopy

Normal white light consists of electromagnetic waves that are oscillating perpendicular to the direction of propagation in all directions. In a transmitted polarized light microscope, two crossed polarizing filters are placed in a light path. The first polarizing filter (the polarizer) is located below the specimen, and only the light waves oscillating in one specific direction pass through it. The light passes through a specimen to the second polarizing filter (the analyzer). Polarized Light Microscopy (PLM) is suitable for investigation of so-called birefringent materials where the refraction of light depends on polarization. The instrument setup has to be standardized to achieve comparable results [36, 50].

Many characteristic features of plant fibres, such as dislocations, crystals, convolutions, and adhering tissue, are enhanced in polarized light, but most importantly, the rotation of the microfibrils in the secondary cell wall, is a birefringent effect which can be identified with the modified Herzog test. The rotation can be right-handed (Z-twist) or left-handed (S-twist). Flax and hemp have opposite twist directions of microfibrils in the S1 sublayer of the secondary layer. In the Herzog test, a so-called red-plate compensator, also called lambda plate, is introduced in the light path, which converts the phase difference induced by the refractive interference difference into a colour difference, and the two different twist directions can be distinguished from each other, which makes that S-twist appears blue (Indigo II) and Z-twist appears orange (Orange I) when oriented in the 0° position and exactly opposite (S-twist orange and Z-twist blue) when oriented in the 90° position.

The Herzog test known since 1920's [43, 51] has been reported in literature [15, 33, 36, 41, 52] and re-examined by a mathematical model recently [53]. It was concluded

that it is one of the easiest and most reliable methods for distinguishing microfibrillar orientation within bast fibres (Haugan and Holst [53]). The test has been demonstrated as an educational video [54]. In 2019 it was established as an ISO standard for distinguishing between flax and hemp fibres [50].

Scanning electron microscopy

In Scanning Electron Microscopy (SEM) an electron beam is produced, focused, and scanned to raster an image or another type of information as e.g., element spectra. The signals are produced from the electron beam—specimen interaction. Scanning electron microscopes reach significantly higher resolution than light microscopes because they are not limited by the wavelength of visible light as optical microscopes. Another advantage is the depth of field that is also much better than in conventional optical microscopes [55]. However, this advantage may lead to a disadvantage for instance misleading cross-section shape and diameter caused by sample preparation.

In SEM, the detected signals come from an incident surface of a sample, which means there is no information on the internal structure of a sample. This is very important to understand since identification features that are inside a fibre structure such as the distinguishing feature of fibrillar orientation cannot be observed by SEM.

The main signals produced are secondary electrons (SE), Back-scattered electrons (BSE), X-rays (EDS), Auger electrons. Secondary electrons are by far the most used imaging signals in SEM for studying fibres ([55], p.51–54). Back-scattered electrons provide information about the sample's elemental composition and their distribution within a sample since the intensity of backscattered electrons depend on the atomic number of the elements in a sample. One of the many advantages of scanning electron microscopy compared to optical microscopy is the higher depth of field, which means that the image of a sample appears sharp over a much more significant height difference than in standard optical microscopy. However, this means that one observes a projected image which distorts the actual dimensions. For instance, a fibre diameter of a perfectly cylindrical fibre will only be presented correctly if the cross-section is parallel to the projection plane; otherwise, the cross-section shape will be wrong, changing from cylindrical to ellipsoidal. Modern Scanning Electron Microscopes are often equipped with a stage offering to tilt in all directions, which can, in some cases, help to solve this problem.

Archaeological specimens may not withstand high vacuum. The problems can be solved with Low-Vacuum SEM or Environmental Scanning Electron Microscopy (ESEM). However, these techniques result in a smaller depth of field ([56], p.413).

A plant fibre identification diagram for flax, hemp, nettle, jute, hops and cotton based on optical and/or electron microscopy

In this section, we present a plant fibre identification diagram (Fig. 4) which can be used to distinguish between flax, hemp, nettle, jute, hops and cotton. The diagram is based on the state of the art of well-founded characteristic- and distinguishing features as presented in the literature. More speculative identification criteria, which have not yet been thoroughly tested, have not been included. Here we refer to the unfounded claim, which can sometimes be observed that cracks in fibres, shown with electron microscopy, can be used to identify the fibrillar orientation [57]. We are not saying that this claim may not be correct, but it has not yet been adequately investigated. We encourage the community to report such observations also in future publications, but for now, not to use them as an identification criterion.

Note that when using this diagram in future publications, it is essential to highlight the fact that it is assumed as a starting point that the fibre is either flax (*Linum usitatissimum*), hemp (*Cannabis sativa*), nettle (*Urtica dioica*), jute (*Corchus olitorius*), hops (*Humulus lupulus*) or cotton (*Gossypium arboreum and Gossypium herbaceum*) or a subsection of this group and to justify why this is a reasonable assumption given the cultural context of the textile material examined. Justifications can include literary sources as well as supporting archaeological finds in the form of, for example, pollen.

Pitfalls

Scientific instruments need to be applied correctly to produce reliable results. It is also essential to know the limitations of the instruments when the results are being interpreted. The state of the sample and sample preparation are also crucial.

Optical microscopy—the modified herzog test

A transmission optical microscope should always be appropriately aligned with Köhler's Illumination before use, Köhler illumination ensures that the light is spread evenly across the sample [36]. This is particularly important for getting a clear image with the Herzog test. It is also important to pick a proper section of fibre to test. Generally, thicker parts of single fibres are most suitable for the test. The ideal fibre section does not have any dislocations (nodes) and/or cross markings that disturb the crystalline structure. Most importantly a focus at the top of the fibre is required for a reliable result [58]. Wrong focusing can lead to the wrong identification, Fig. 5.

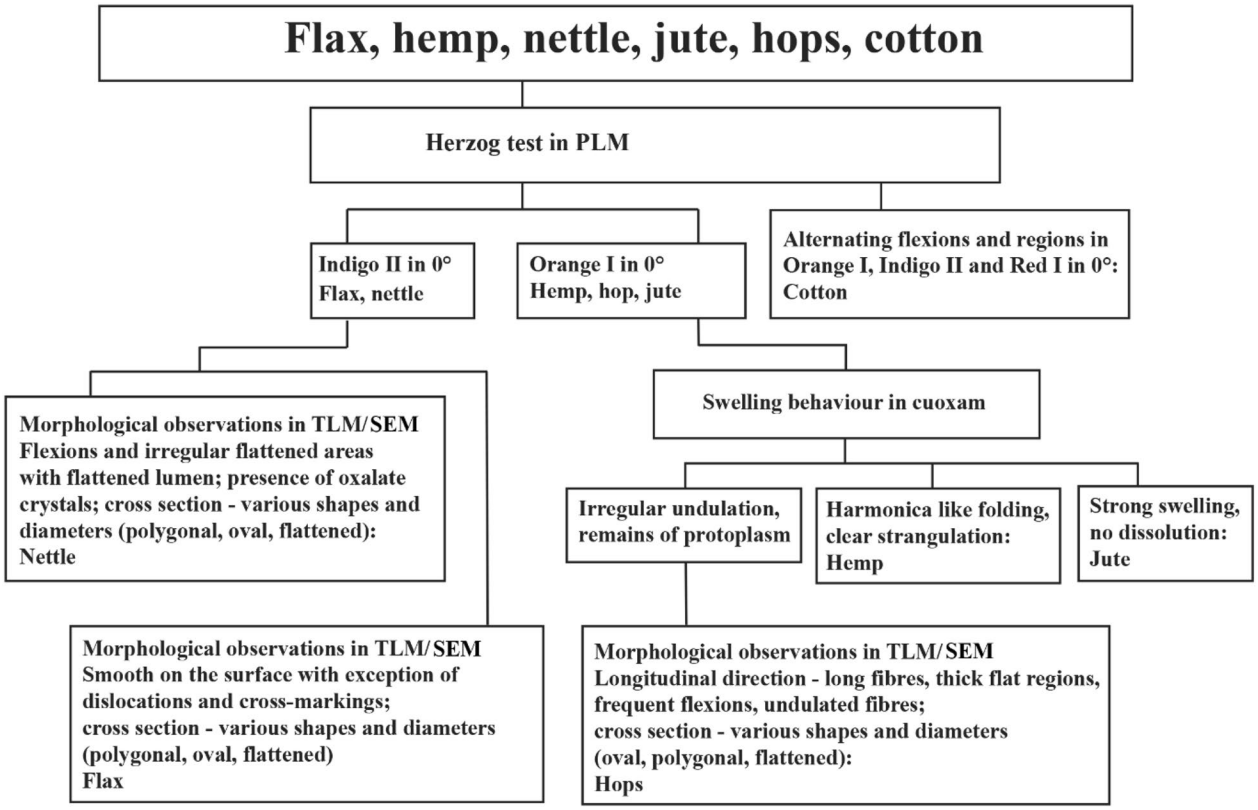

Fig. 4 Diagram for distinguishing between flax (*Linum usitatissimum*), hemp (*Cannabis sativa*), nettle (*Urtica dioica*), jute (*Corchus olitorius*), hops (*Humulus lupulus*) and cotton (*Gossypium arboretum*) by means of optical microscopy, © Lukesova

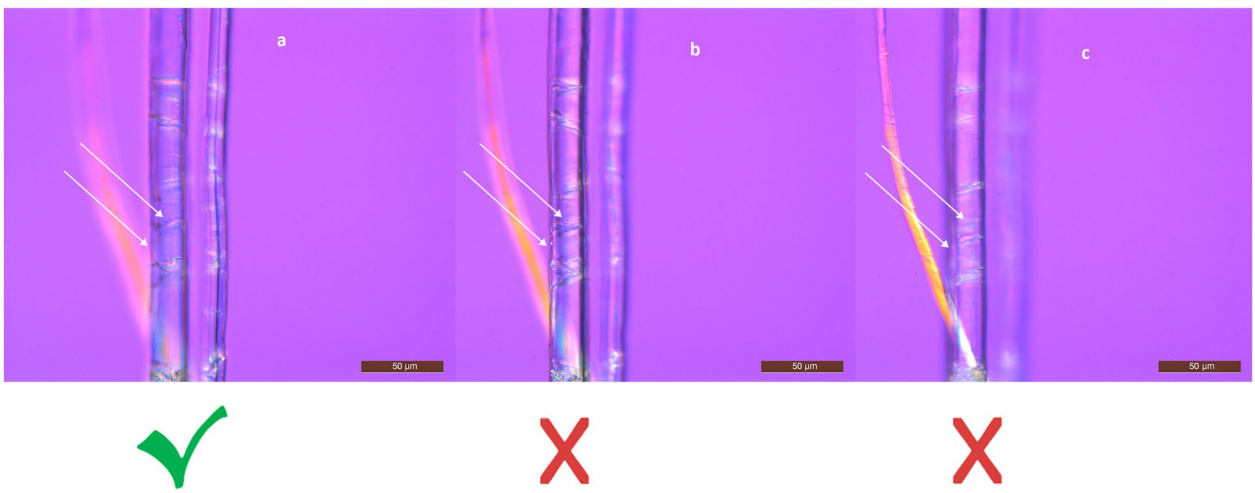

Fig. 5 **a** The proper focus on the fibre's top is crucial for the correct interpretation of interference colours of the modified Herzog test. The arrows show the differences in the focus on the fibre's top and its edge. Figures **b** and **c** have the focus point underneath the top of the fibre. © Lukesova

Sample state and sample preparation

Fibre analysis of heritage material is, in many ways, different from the study of modern fibres. This is often overseen, and researchers tend to apply procedures developed for the textile industry even though such procedures are not always appropriate.

The path to a reliable result starts already before sampling a studied object. Necessary knowledge on a

macroscopic level is a must, together with a clear strategy regarding the research aim. This may sound obvious, but careful planning of sampling and sample preparation is crucial for a successful result since any sampling inevitably narrows down the focus from a whole object to a specific object area. A sample must be representative of an object and for a research question. The choice of such a place is essential for later investigation.

Cultural heritage objects are unreplaceable. It is necessary to consider the need for the research and to consider possible harm to a studied object. Many museums follow ICOM's ethical guidelines regarding treating cultural heritage [59]. A sampling of a cultural heritage object must be performed with the highest caution, documentation and use of appropriate tools like fine tweezers and surgical scissors.

Sample preparation for optical microscopy

Sample preparation requires concentration, slow breathing, a stereo- or digital microscope, ultra-fine tweezers, and a tungsten needle. A tungsten needle is a useful preparation instrument with a very pointed, slightly charged tip allowing small particles to cling to the needle with electromagnetic forces only. It is easy to pick up tiny particles and then remove them from the needle with a smooth rotation movement. Such needles can either be purchased from special suppliers of laboratory equipment, or they can be prepared from a tungsten wire [36].

Knowledge about refractive indices of sample materials and mounting media is important in transmitted light microscopy. Refractive index of a material (n_D) is a dimensionless number expressing the ratio of the speed of light in a vacuum to the speed of light in that material. The difference between refractive indices of a transparent object and its surrounding medium is crucial for the object's visibility. For instance, a gel bead ($n_D \approx 1{,}33$) surrounded by air ($n_D \approx 1{,}00$) is well visible since the difference of the refractive indices is big enough to achieve a sufficient phase contrast (Fig. 6a). If the same bead is half sunk in water—only its upper part, surrounded by air is visible (Fig. 6b). The bead is not visible, when sunk in water completely because refractive indices of the bead, and water are too similar (Fig. 6c). Two coloured beads and one transparent bead are surrounded by air and thus

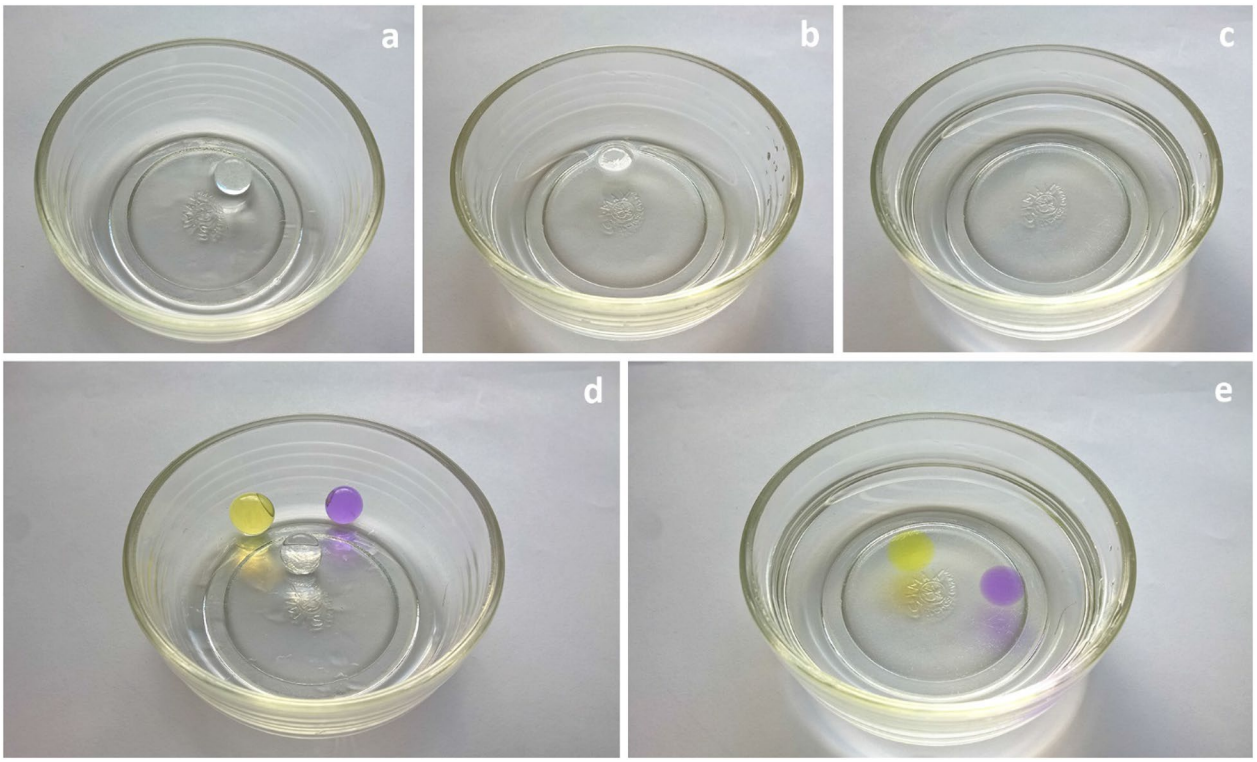

Fig. 6 The difference between refractive indices of a transparent object and its mounting medium is crucial for the object's visibility: **a** Left above: A gel bead is surrounded by air; **b** middle above: the same bead is half sunk in water—only its upper part, which is surrounded by air is visible; **c** right above: the same bead is completely sunk in the water and is not visible, because refractive indices of the bead and water are too similar. **d** Bottom left: Two coloured beads and one transparent bead are surrounded by air. **e** Bottom right: The three beads are completely sunk in water—only the two coloured ones are visible with blurred edges. The edges are blurred because there is only colour contrast and not phase contrast, © Lukesova

well visible (Fig. 6d). Only the two, coloured beads are visible with blurred edges and the third one disappeared. They are completely sunk in water and the edges of the coloured ones are blurred because there is only colour contrast and not a phase contrast (Fig. 6e) [58].

Thus, for transmitted light microscopy investigations, the choice of mounting media is essential because the difference in refractive indices of a mounting medium and a studied object ($\Delta n = n_{D1} - n_{D2}$) influences the object's visibility as a phase contrast. The Fig. 6e demonstrates that staining of transparent samples helps to enhance the contrast, but it cannot substitute the proper choice of mounting medium.

Sample preparation for scanning electron microscopy

Usually, a specimen must be dry and conductive since the specimen chamber is at a high vacuum. A specimen is placed on an aluminium stub covered with colloidal silver or graphite. Today, special double-sided conductive tapes are also available. A specimen is coated with carbon (C) or gold (Au) particles dependent on the grain size needed (Au has a smaller grain size than C. Platinum/palladium (Pt/Pd) or Au/Pd is used to obtain even a smaller grain size than Au. Several methods can be used for coating as sputter coating or metal evaporation.

Documentation tables for optical- and electron microscopy measurement settings

A scale bar should always be included in a microscopy image to show the resolution. It is not enough to state the magnification since that will change according to the size of the image. Documentation tables for all the microscopy images shown in this paper can be found in the supplementary material section (Table 2 and 3).

Optical microscopy

Scanning electron microscopy

Most fibre identification images are made using secondary electron detection because this is typically used for general imaging, as it is most sensitive to topography. A backscattered electron detector is more sensitive to the chemical composition of a sample. The images look very different (Fig. 7). Flax fibres are presented with the backscattering detector, BSE (Fig. 7a) and with the secondary

Table 3 Recommended specifications for Scanning Electron Microscopy measurements on heritage fibres

Specification no	Technical specification	Comment
1	Manufacturer and instrument model	For documenting the quality of the instrument
2	Acceleration voltage	
3	Working distance	
4	Beam current	
5	Resolution (spot size)	
6	Detector type used	SE, BSE
7	Make, and serial number of sample coater used	For documenting the quality of the sample preparation
8	Sample coating material	No coating, C, Au

Table 2 Recommended specifications for Optical Microscopy measurements on heritage fibres

Specifi-cation no	Technical specification	Comment
1	Manufacturer and instrument model	For documenting the quality of the instrument
2	Light source	What light source has been used with what settings?
3	Oculars	
4	Objective	Manufacturer, Lateral Magnification, Numerical Aperture, Immersion Medium, Flat-Field Correction, Aberration Correction, Specialized Optical Properties, requested Tube Length, requested Coverslip Thickness, Working Distance
5	Condensor	Manufacturer, Magnification, Numerical Aperture
6	Köhler illumination	Has the instrument been adjusted for Köhler illumination after each objective change?
7	Camera, its mount and the software	What camera has been used and what were the settings?
8	Mounting medium	Type and value of refractive index
9	Cover slip: Thickness and material	Each objective Aperture tolerates a specific coverslip thickness and its deviation of Cover glass Thickness; the material influences the refraction of light in the light path
10	Operation mode	E.g. Bright Field Polarized Light Microscopy Phase Contrast

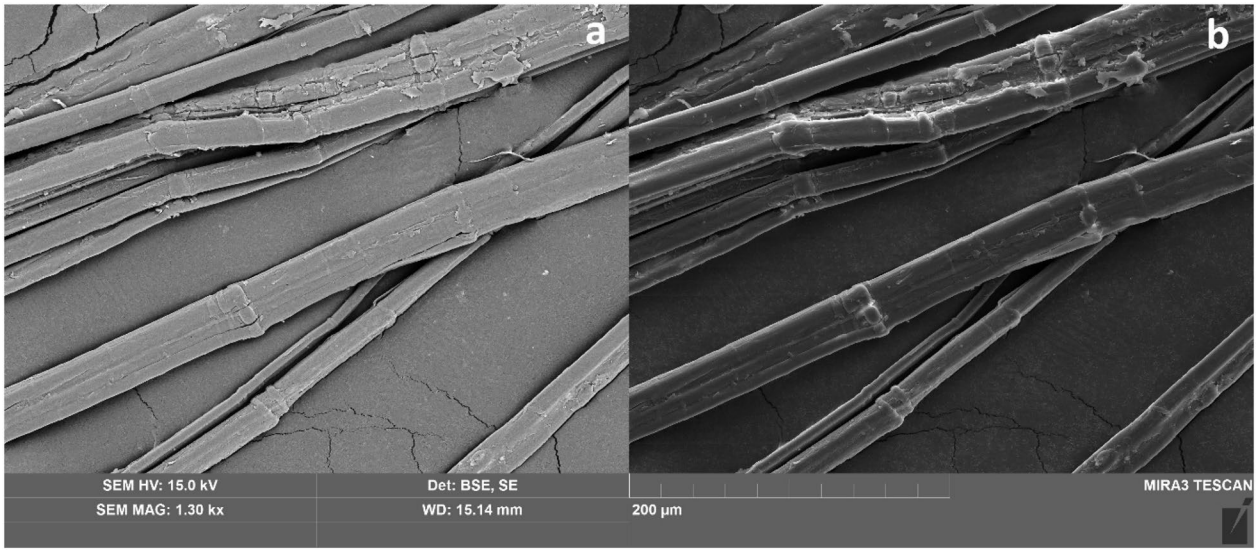

Fig. 7 Flax fibres imaged with BSE detector (on the left) and SE detector (on the right), © Lukesova

detector, SE (Fig. 7b). The BSE detector provides information about the sample's elemental composition and distribution and is thus less useful for fibre identification than SE, which is commonly used for imaging of surface structures of biological specimens. Characteristic features as dislocations on herbaceous bast fibres can be well visualised by SEM-SE (Fig. 7b).

Demonstration example of fibre identification analysis with documentation tables of the 10th Century Viking age burial textiles

Here we present an example of application the documentation tables for optical microscopy.

An archaeological textile find (fragments of plain weave—tabby) belonging to the 10th Century Viking Age burial (University Museum of Bergen, B 4864_g,h) was studied (Fig. 8). The textile remains were interpreted as part of a woman's shift based on the reconstruction of microstratigraphy oof textile layers belonging to the oval brooches B 4864 i that were found in the same grave [28, 60].

The fibre identification was performed under the assumption that the textile is made by either flax, hemp, or nettle. This assumption is based on the archaeological context. It can be debated and should therefore always be made clear at the beginning of the analysis as discussed

Fig. 8 Viking Age textile remains interpreted as remains of woman's shift (**a**, on the left); Viking Age oval brooches used as a functional decoration of the so-called suspended dress (**b**, on the right)

above. Under this assumption the identification diagram in Fig. 4 can be used. The identification procedure was done on 50 single fibres showing the same result. Ideally more than 100 fibres should have been investigated to comply with the ISO standard [50], however, this was considered too much based on the limited amount of material available. The modified Herzog Test confirmed an Indigo II in 0° position (Fig. 9c) and Orange I in 90° position (Fig. 9d) that stem for a feature distinguishing flax and nettle from hemp (as well as jute, hops and cotton, which are not relevant for the archaeological context). Figure 9a–d are followed by the tables documenting the technical specifications of the measurements (Table 4). Morphological observations in Transmitted white light show that all fibres displayed characteristic flax features such as a smooth fibre surface, with dislocations and cross-markings. Features characteristic for nettle: flattened areas, flexions and/or oxalate crystals were not present in any of the 50 fibres examined. Based on the analysis we propose that the fragments are very likely made of flax.

Conclusions and outlook

Identification of fibres by means of microscopy is indispensable in modern research on cultural heritage and conservation. Various techniques, such as transmitted white light-, polarized light- and scanning electron microscopy, have been discussed. The sub-discipline of fibre microscopy on cultural heritage is a vivid discipline on the rise of deserving joint forces. Microscopy, as such, has excellent potential for future cultural heritage studies and its application in the field of textile conservation. The sub-discipline requires adaptation of

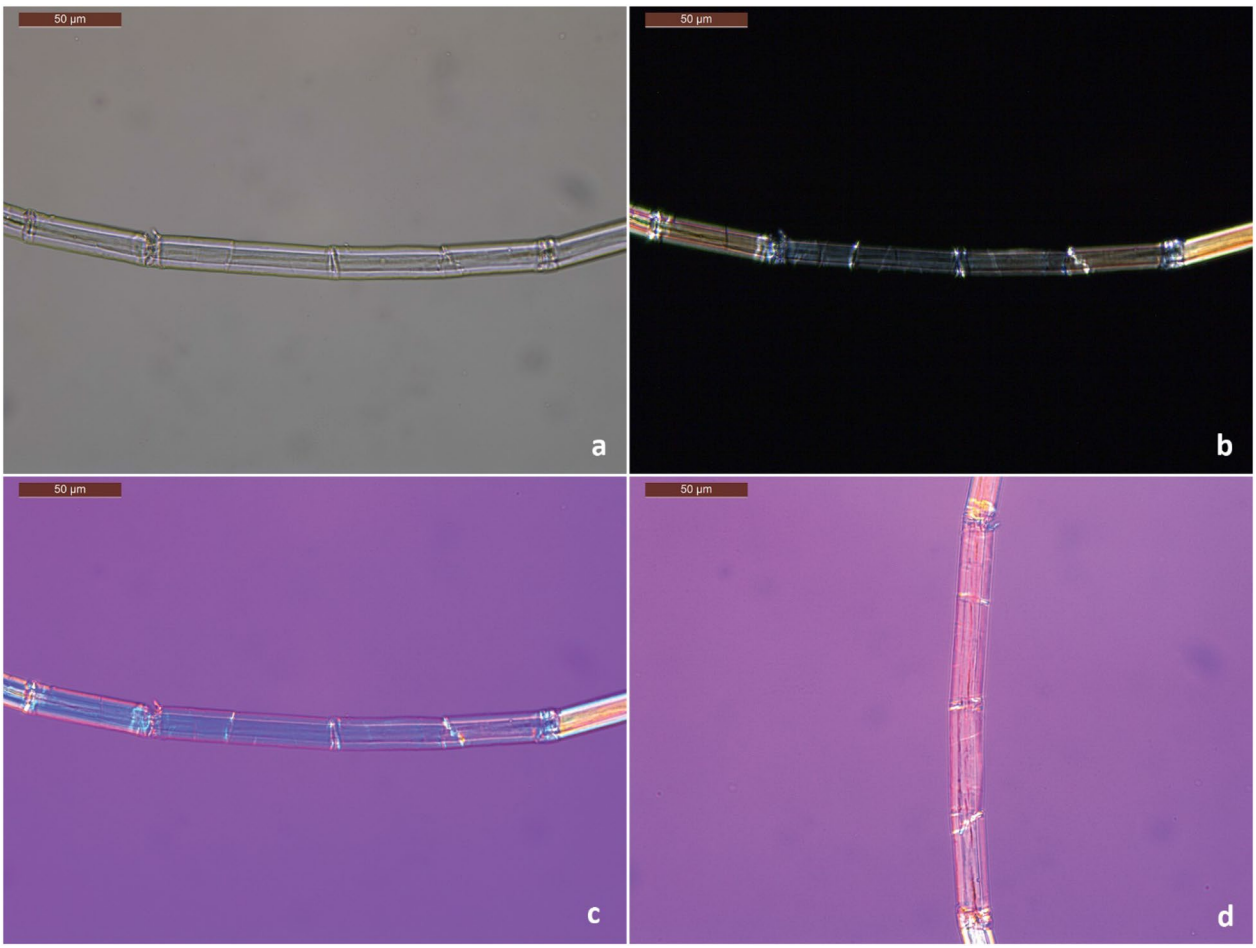

Fig. 9 Transmitted white light- (**a**) and Polarized Light Microscopy PLM (**b**, **c**, **d**) of archaeological textile find B 4864_g,h belonging to the 10th Century woman's shift from the Viking Age burial. **a** Transmitted white light image showing smooth fibre surface with dislocations and cross-markings. **b** PLM with crossed polars showing almost complete extinction in the middle of the image—an area suitable for performing the Modified Herzog Test. **c** PLM with crossed polars and red-plate compensator, the fibre shows Indigo II in 0° position. **d** PLM with crossed polars and red-plate compensator, the fibre shows Orange I in 90° position

Table 4 A documentation table of measurement settings for Fig. 9a–d

Specifi-cation no	Technical specification	Comment
1	Manufacturer and instrument model	Leica DM 750P
2	Light source	LED
3	Oculars	Leica HC PLAN s10x/20
4	Objective	HI PLAN, 40x/0,65 POL; dry; ∞/0,17/OFN25
5	Condensor	CLP/PH 0,85 S1
6	Köhler illumination	Yes
7	The camera, its mount and the software	Leica MC170 HD; C-mount 0,55x; LAS V4,13
8	Mounting medium	Meltmount ® $n_D = 1662$
9	The thickness of the coverslip glass	0,17 mm
10	Operation mode	Figure 9a: BF Figure 9b: PLM—Crossed polars, 0° sample orientation Figure 9c: PLM—Crossed polars, red-plate compensator, 0° sample orientation Figure 9d: PLM—Crossed polars, red-plate compensator, 90° sample orientation

methods on the unique and irreplaceable materials due to the following reasons:

1. Not only the species used as commercial fibres were used for textile production throughout history. 2 Historical processing methods that differ from modern ones may impact the fibre's appearance, 3 The material of historical and archaeological objects is often degraded, which requires specific knowledge related to sampling and interpreting of results and limits the methods that can be used (i.e. carbonization prevents the use of transmission light microscopy). 4 Working with cultural heritage material raises ethical issues regarding the number and the size of core samples, which leads to limitations in terms of a possible number of sub-samples and the use of statistical evaluation of data. This means that fibre identification of cultural heritage material should strictly differentiate between characteristic and distinguishing features, and the main emphasis in this sub-discipline should go towards further research on distinguishing features—ideally on historical reference samples or at least on artificially aged modern reference samples.

Abbreviations

SEM	Scanning electron microscopy
SEM-SE	Scanning electron microscopy with secondary electrons detector
SEM-BSE	Scanning electron microscopy with backscattered electrons detector
µXRD	X-ray micro-beam diffraction
FTIR	Fourier transform infrared spectroscopy
aDNA	Ancient deoxyribonucleic acid
TLM	Transmitted light microscopy

Supplementary Information

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Additional file 1: Figure S1. a SEM micrograph of sheep wool. b SEMmicrograph of cotton fibres with clear convolutions. **Figure S3.** a Nettle fibre (*Urtica dioica*) showing flattened area, transmitted white light microscopy. b Nettle fibre (*Urtica dioica*) showing clear flexion, transmitted white light microscopy. **Figure S7.** a SEM micrograph of flax fibres. b SEM micrograph of flax fibres.

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Declarations

Competing interests
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Publisher's Note

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