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Textiles — Qualitative and quantitative analysis of some bast fibres (flax, hemp, ramie) and their blends —

**Part 1:
Fibre identification using microscopy methods**

Textiles — Analyses qualitative et quantitative de certaines fibres libériennes (lin, chanvre, ramie) et de leurs mélanges —

Partie 1: Identification des fibres à l'aide de méthodes microscopiques

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 38, *Textiles*.

A list of all parts in the ISO 20706 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

This corrected version of ISO 20706-1:2019 incorporates the following corrections:

- in [8.3](#), the SEM procedure structure has been corrected;
- in [7.2.1](#), the missing reference to [Annex H](#) has been added.

Introduction

Among bast fibres used for textiles, flax and hemp are the most expensive. Flax is grown mainly (85 %) in a small coastal area of Northern Europe; hemp textile products are rare. Ramie is less expensive than flax and hemp: 10 % to 20 % cheaper for medium count yarns — and the difference increases for fine counts.

Flax and other bast fibres, such as hemp and ramie exhibit great similarities in their physical and chemical properties, so that their fibre specie and their blends are difficult to distinguish from each other by both mechanical and chemical methods. In addition, these fibres show nearly resembling fibre morphology. It is very difficult to accurately identify the fibre species and accurately determine the fibre content of such fibre blends by current testing means.

Research works on accurate identification of bast fibre has been a long undertaking.

In order to promote fair labelling of products and anti-counterfeiting protection, The European Confederation of Flax and Hemp (CELC) created the Bast Fibre Authority in 2013, inviting laboratories, research centres and providers of quality and control services to develop a common technical protocol. Five laboratories joined in 2013 and comparison testing were carried out between them on May–June 2014 and January–February 2015.

NOTE CELC, founded in 1951, is a non-profit organization and an association for reflection, market analysis, industry concertation and strategic orientations. CELC is the only agro-industrial European organization that covers all stages of production and processing of flax/linen and hemp. It is the chosen representative of more than 10,000 firms in 14 European countries, promoting the fibre from plant to finished product (including sections dealing with agriculture, retting/scutching, trading, spinning, weaving and technical uses).

At present, the most widely used and reliable ones include light microscopy (LM) method and scanning electron microscopy (SEM) method. The advantage of LM method is that the internal morphology of fibres can be observed, but some subtle surface structures are not able to be clearly displayed. Decoloration process can be carried out on dark samples for testing, while improper decoloration process will affect the judgment of fibre analyst.

The scanning electron microscopy (SEM) method shows opposite characteristics to those of LM method. Therefore, some types of fibres need to be identified by scanning electron microscope.

When some samples are difficult to be identified, light microscopy method and scanning electron microscopy method should be used together to identify in order to utilize the advantages of both methods.

It is proven in practice that accuracy of fibre analysis is highly related to the ample experience, fully understanding and extreme familiarity of the fibre analyst to the morphology of various types of bast fibres. Therefore, besides text description, a large amount of micrographs of different types of flax, hemp and ramie are given in [Annex A](#), [Annex B](#), [Annex C](#) and [Annex D](#) of this document.

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Textiles — Qualitative and quantitative analysis of some bast fibres (flax, hemp, ramie) and their blends —

Part 1: Fibre identification using microscopy methods

1 Scope

This document specifies methods for the identification of some bast fibres (flax, hemp, ramie) using both light microscopy (LM) and scanning electron microscopy (SEM). This document is also applicable to blends of these bast fibres and products made from them.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 20705:2019, *Textiles — Quantitative microscopical analysis — General principles of testing*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 20705 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at <https://www.iso.org/obp>

— IEC Electropedia: available at <http://www.electropedia.org/>

3.1

bast fibre

composite fibre obtained from the bast of certain plants, mainly constituted of cellulose and accompanied with incrusting and intercellular materials (pectin bodies, hemicellulose, lignin)

Note 1 to entry: Adapted from ISO 6938:2012, 2.3.

3.2

flax

fibre from the basts of flax *Linum usitatissimum*

Note 1 to entry: Adapted from ISO 6938:2012, 3.2.2.5.

3.3

hemp

fibre from the basts of *Cannabis sativa*

Note 1 to entry: Adapted from ISO 6938:2012, 3.2.2.1.

3.4

ramie

fibre from the basts of ramie *Boehmeria nivea*, *Boehmeria tenacissima*

Note 1 to entry: Adapted from ISO 6938:2012, 3.2.2.6.

3.5

technical fibre

assembly of ultimate fibres (usually 20 to 40 ultimate fibres) as it occurs in the plant after extraction (mechanical, chemical, etc.)

3.6

ultimate fibre

unitary fibre obtained from a fibre bundle after removal of non-cellulosic components including pectins

3.7

lumen

canal in the centre of the fibre where are located the cells and organites, surrounded by the plasma-membrane and cell walls

3.8

knot

cell walls deformations with changes in the chemical or/and physical structures which have been formed either during the growth of the plant or during processing forming like a ring around the fibre

3.9

pit

cavity in the lignified cell walls of xylem conduits (vessels and tracheids) that are essential components in the water-transport system of higher plants

Note 1 to entry: The pit membrane, which lies in the centre of each pit, allows water to pass between xylem conduits but limits embolism and the spread of vascular pathogens in the xylem.

3.10

crack

structural default induced during mechanical process (e.g. scutching, cross section cutting)

3.11

longitudinal striate

structural appearance along the fibre due to fibre shape ridges

3.12

test specimen unit

linear portion of a single thread

[SOURCE: ISO 20705:2019, 3.1]

4 Principle

A longitudinal view image and/or cross view image of fibres representative of a test specimen is magnified to an appropriate scale/size under optical light microscope and/or SEM. All bast fibre species found in the test specimens are identified by the difference in fibre morphology among different types of certain bast fibres (flax, hemp, ramie).

5 Apparatus

Use the apparatus described in ISO 20705, together with those described in [5.1](#) and [5.2](#).

5.1 Transmitted-light type microscope (described in ISO 20705:2019, 5.1), shall be capable of providing a magnification of $\times 250$ to $\times 500$. The focal of this type of microscope shall be capable of discriminate details up to $2\ \mu\text{m}$ to $3\ \mu\text{m}$; therefore, a magnification factor of at least $\times 400$ is recommended.

The transmitted-light type microscope shall comprise:

- a light condenser that includes a diaphragm based on Köhler illumination to obtain image with high resolution;
- a polarized device (i.e. polarizer and analyser) with a retardation plate of $530\ \text{nm}$ (known as “red plate”).

5.2 Visual microscopic image analyser, shall comprise a microscope, a camera, a computer, a data acquisition card, exclusive analysing software and a display. The objective and ocular of the microscope shall be capable of providing at least a magnification of $\times 500$. The focal of this type of microscope shall be capable of discriminate details up to $2\ \mu\text{m}$ to $3\ \mu\text{m}$.

6 Reagents

Use the reagents described in ISO 20705, together with reagents described in [6.1](#) and [6.2](#).

6.1 Water, Grade 3 according to ISO 3696.

6.2 (Clear) glycerine.

7 Sampling

7.1 Laboratory sample

Principles of the selection of the laboratory sample shall be according to [Annex H](#).

7.2 Preparation of the test specimens

7.2.1 Selection of the test specimens

Select the test specimens as described in ISO 20705, together with selection described in [Annex H](#) for finished products. Select sub-samples (e.g. fabrics or yarns) representative of the materials used in the finished products and then select and prepare the test specimen as described in ISO 20705.

7.2.2 Preparation of a test specimen

Follow the test specimen preparation for cross view described in ISO 20705, together with those described specifically for longitudinal view below.

Instead of snippet cuttings, put basically parallel fibres from the fibre bundle or the test specimen unit (by untwisting) on the LM slide (in 2 places) or, if required, on the SEM stub (then, in this case, prepare a duplicate stub).

The specific preparation for longitudinal view on SEM may be carried out if required because it is worthless as SEM longitudinal views of flax, hemp or ramie are similar and do not lead to give clues to differentiate these bast fibres (see [Annex D](#)).

8 Procedure

Follow the general procedure described in ISO 20705, and then proceed as follows.

8.1 General

Carry out both longitudinal view and cross view procedures, except when only ramie is identified (by cross view preferably – or longitudinal view), using either LM and/or SEM.

NOTE 1 For justification of this instruction, see [Annex D](#).

Identify the fibre species, based on the comparative fibre morphology attributes of flax, hemp and ramie, as listed in [Table 1](#).

NOTE 2 The fibre morphology attributes are based on ultimate fibres.

Record the pictures of the identified fibres.

At least 100 fibres shall be identified.

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Table 1 — Comparative fibre morphology attributes of flax, hemp and ramie

POINTS OF INTEREST	CHARACTERISTICS	VIEW	FLAX (see photomicrographs in Annex A)	HEMP (see photomicrographs in Annex B)	RAMIE (see photomicrographs in Annex C)
Knot	External morphology	Longitudinal	Knot diameter is mostly greater than the fibre body diameter. Knots cover the whole fibre diameter.	Knot diameter is as large as fibre body. Knots may frequently not cover the whole fibre diameter.	Knot diameter is as large as fibre body. Knots may frequently not cover the whole fibre diameter. Knots present some disorientation (due to helicoidal path) into V shape.
			Knots are frequent on one portion (but knots may be absent on some portions)	There is presence of knots but less frequent than flax	There is presence of knots but less frequent than flax
Pit	Pit presence	Longitudinal	(only LM) Pits are less frequent, less visible.	(only LM) Pits with medium frequency	(only LM) Pits are very frequent and visible and oriented in all directions, often in V, Y or X shapes
Fibre surface	Longitudinal striates	Longitudinal	The polygonal shape of the ultimate fibre leads to get few longitudinal striates. (only LM) Note that they are not to be confused with the lumen.	The polygonal shape of the ultimate fibre leads to get few longitudinal striates. (only LM) Note that they are not to be confused with the lumen.	Due to circumvolutions of the surfaces, there is a presence of numerous longitudinal striates. (only LM) Note that they are not to be confused with stronger lines due to irregular shapes (U, Z...).
			No twisting visible if the fibres have a small lumen and are full mature. If the fibres have not reached their maturity and have a large lumen, a longitudinal twist is nonetheless possible	No twisting visible if the fibres have a small lumen and are full mature. If the fibres have not reached their maturity and have a large lumen, a longitudinal twist is nonetheless possible	Fibre may be twisted, due to transformation during spinning, occurring on some flattened ramie fibres [not visible on raw fibres before spinning]
Overall shape	Morphology	Longitudinal	Pentagonal shape with small lumen if mature fibre. Non mature fibres are flattened and have larger lumen. The corners of the pentagonal shape are generally a bit sharper than hemp.	Pentagonal shape with small lumen if mature fibre. Non mature fibres are flattened and have larger lumen. The corners of the pentagonal shape are generally a bit more rounded than flax	Most fibres have a flattened shape and have a large lumen.
		Cross			

Table 1 (continued)

POINTS OF INTEREST	CHARACTERISTICS	VIEW	FLAX (see photomicrographs in Annex A) (only LM) Lumen is visible.	HEMP (see photomicrographs in Annex B) (only LM) Lumen is visible if ultimate fibre.	RAMIE (see photomicrographs in Annex C) (only LM) In relation to the fibre orientation, some lumens are visible and wider.
Lumen		Longitudinal Cross	Lumen of a mature fibre is mostly very small and circular while lumen of a non mature fibre is wider and follows the flattened shape of the fibre	Lumen of a mature fibre is mostly very small and circular while lumen of a non mature fibre is wider and follows the flattened shape of the fibre	Lumen of a mature fibre is mostly wider than flax or hemp lumen and follows the flattened shape of the fibre. From the outer wall of the fibre to the wall of the lumen some cracks may occur
Cracks	Crack presence	Cross	Not many cracks are visible in the cross section.	Not many cracks are visible in the cross section.	From the outer fibre wall to the inner lumen wall a ramie fibre can have some cracks which are probably linked to the longitudinal striates.
Ultimate fibre versus technical fibre		Longitudinal Cross	Technical fibres (as groups of ultimate fibres which are stucked to each other by pectins and other parts of the bast), if in form of bundles, are present in non treated flax Ultimate fibres are present if the flax has been treated (e.g. by caustification, bleaching and colouring). There are no pectins or other parts of the bast.	As hemp has long fibres, there are mostly technical fibres in yarn which are not treated (i.e caustified or bleached). Ultimate fibres are present in treated yarn (i.e. caustified and bleached) and are free of pectins and other parts of the bast.	Technical fibres (as groups of ultimate fibres which are stucked to each other by pectins and other parts of the bast), if in form of bundles, are present in non treated ramie Ultimate fibres are present if the ramie has been treated (e.g. by caustification, bleaching and colouring). There are no pectins or other parts of the bast.
Fibrillar orientation	Birefringence Twist behaviour when drying	Longitudinal Not applicable	Distinction between Flax and Hemp fibres. See Annex E	Distinction between Flax and Hemp fibres. See Annex F	Not necessary Not necessary

8.2 LM procedure

8.2.1 Longitudinal view

Apply the procedure as described in ISO 20705:2019, 8.2.1, together with the following instruction.

When starting the examination of a targeted fibre, move the slide in order to find out any comparative fibre morphology attributes (see [Table 1](#)) along the targeted fibre.

If necessary, when dyed fibres are difficult to be analysed, a fibre decolouration may be carried out (see [Annex G](#)).

Within the purpose to distinguish flax and hemp:

- examine fibres using polarized light (“modified Herzog test”), according to [Annex E](#); and
- process fibres to the “twist test”, according to [Annex F](#).

8.2.2 Cross view

Apply the procedure as described in ISO 20705:2019, 8.2.2.

8.3 SEM procedure

8.3.1 Cross view

Apply the procedure as described in ISO 20705:2019, 8.3.2

8.3.2 Longitudinal view (optional)

Apply the procedure as described in ISO 20705:2019, 8.3.1, together with the following instruction.

When starting the examination of a targeted fibre, move the stub in order to find out any comparative fibre morphology attributes (see [Table 1](#)) along the targeted fibre.

9 Test report

The test report shall include the following information:

- a) a reference to this document, i.e. ISO 20706-1:2019;
- b) the nature of the sample (e.g. fibre, yarn, swatch of fabric, readymade product);
- c) the identification of the sample (e.g. lot number, article number);
- d) the method of sampling;
- e) the number of test specimens;
- f) the type of apparatus: LM or SEM;
- g) the species of identified fibres, with associated pictures;
- h) any deviation from the given procedure.

Annex A (informative)

Photomicrographs of flax (Light microscopy and SEM)

A.1 Photomicrographs of flax by light microscopy

A.1.1 Longitudinal view



Figure A.1 — Longitudinal view of ultimate flax (LM)



Figure A.2 — Knots — Longitudinal view of ultimate flax (LM)

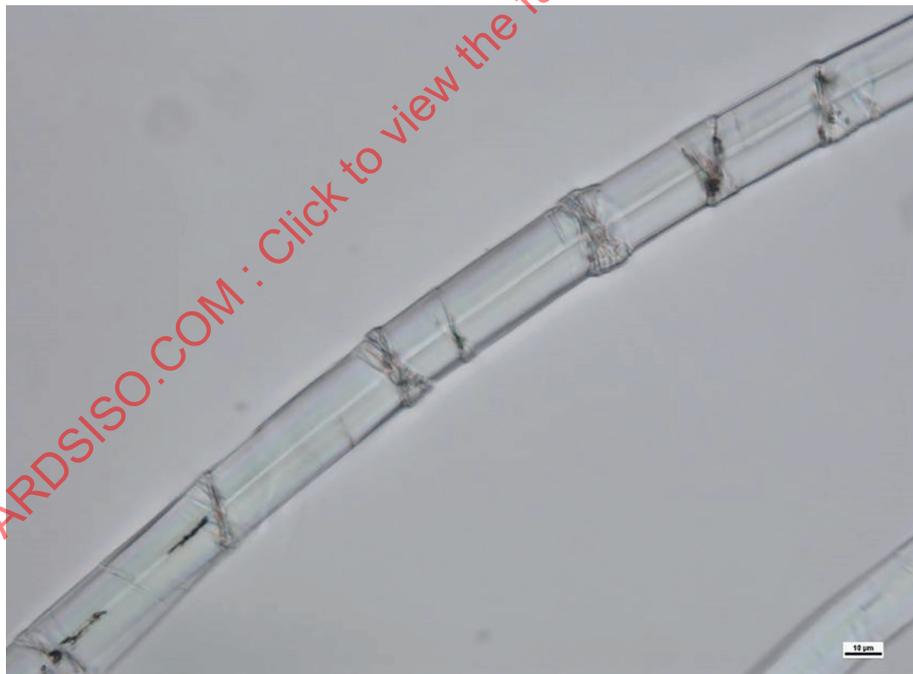


Figure A.3 — Knots and lumen — Longitudinal view of ultimate flax (LM)

A.1.2 Cross view



Figure A.4 — Technical fibres (bundle “a”) — Cross view of untreated flax (LM)

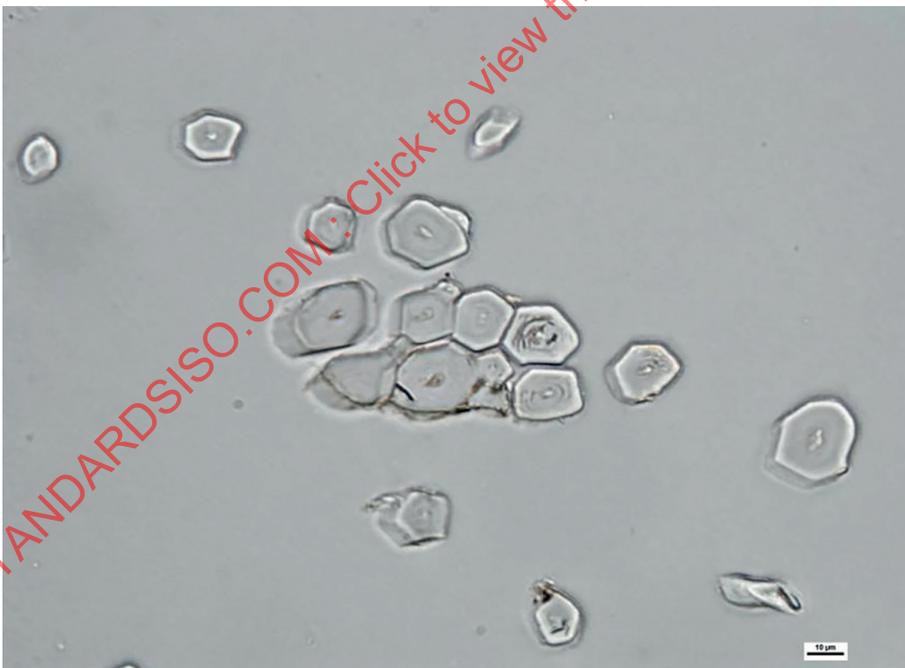


Figure A.5 — Technical fibres (bundle “b”) — Cross view of untreated flax (LM)

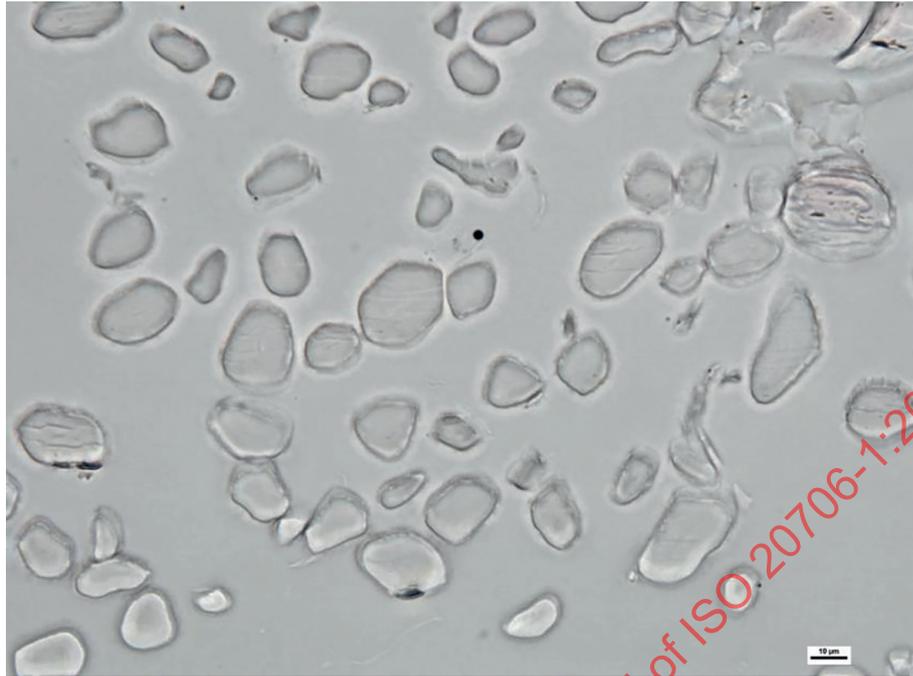


Figure A.6 — Ultimate fibres — Cross view of flax (LM)

A.2 Photomicrographs of flax by SEM (cross view)



Figure A.7 — Pentagonal shape, lumen — Cross view of flax (SEM)

Annex B
(informative)

Photomicrographs of hemp (Light microscopy and SEM)

B.1 Photomicrographs of hemp by light microscopy

B.1.1 Longitudinal view



Figure B.1 — Longitudinal view of hemp (LM)

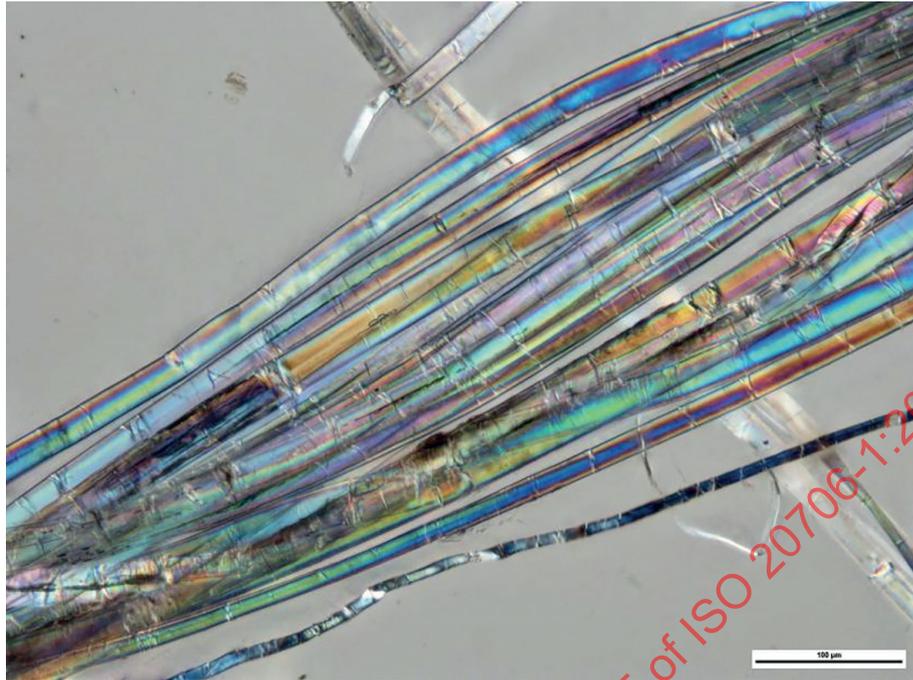


Figure B.2 — Longitudinal view of hemp (LM under polarized light)



Figure B.3 — Knots, lumen of ultimate fibre — Longitudinal view of hemp (LM)

B.1.2 Cross view



Figure B.4 — Technical fibre — Cross view of hemp (LM)

B.2 Photomicrographs of hemp by SEM (cross view)

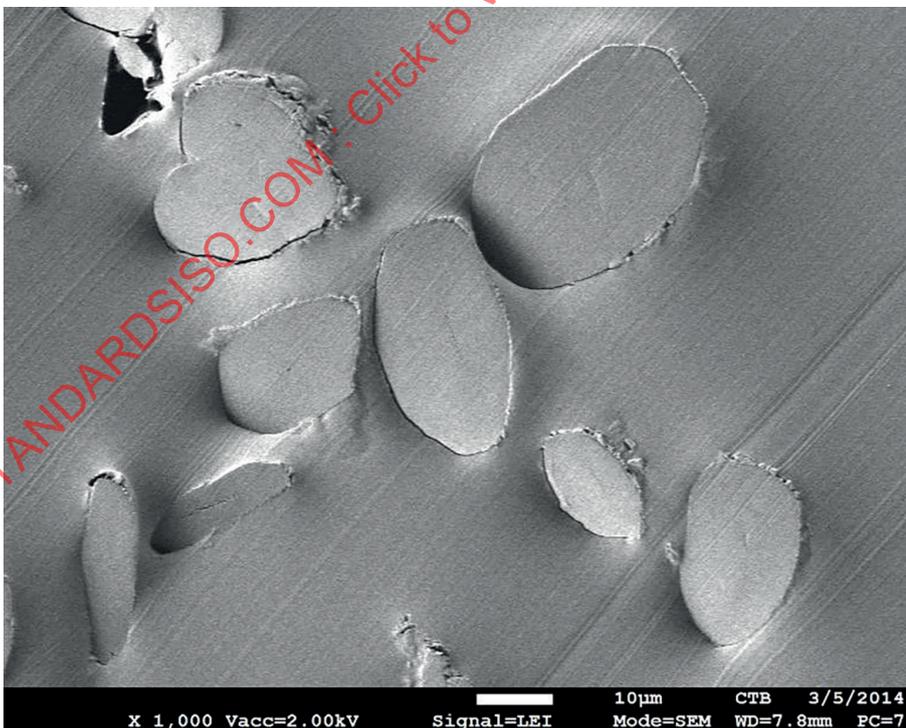


Figure B.5 — Cross view of hemp (SEM)

Annex C (informative)

Photomicrographs of ramie (Light microscopy and SEM)

C.1 Photomicrographs of ramie by light microscopy

C.1.1 Longitudinal view

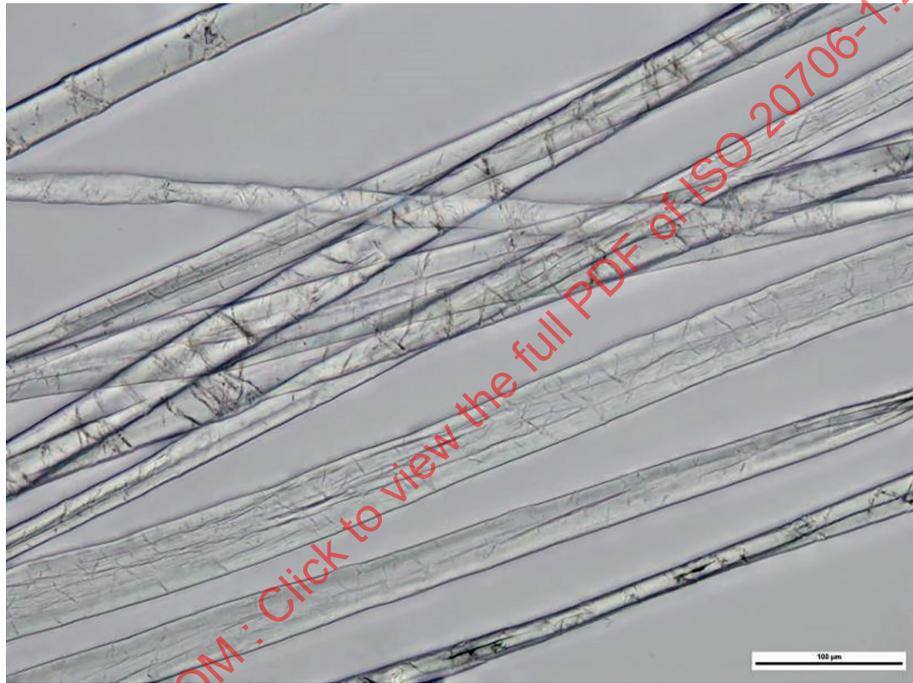


Figure C.1 — Knots, pits, longitudinal striates — Longitudinal view of ramie (LM)



Figure C.2 — Longitudinal striates, fibre twist — Longitudinal view of ramie (LM)

C.1.2 Cross view



Figure C.3 — Cross view of ramie (LM)

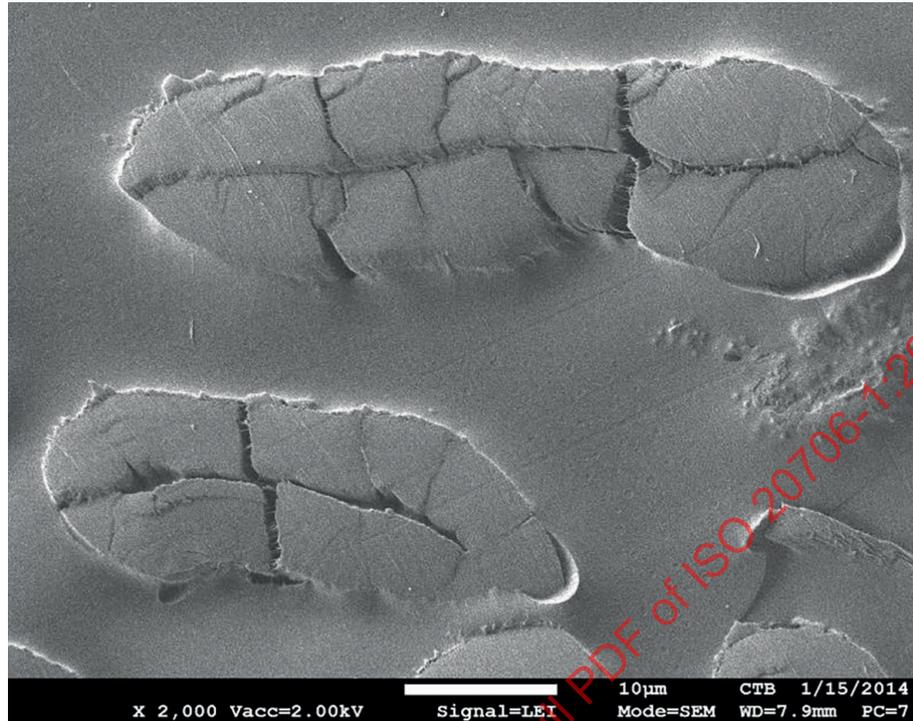
C.2 Photomicrographs of ramie by SEM (cross view)

Figure C.4 — Cracks, lumen — Cross view of ramie (SEM)

Annex D (informative)

Rationale

D.1 General

In 2013 and 2014, CELC organized comparison studies with some European laboratories in order to identify the possible improvements of the identification of some bast fibres such as flax, hemp and ramie (see Introduction).

The first findings about the techniques carried out by these laboratories were that either LM or SEM are used, and that some results were based on longitudinal view and/or cross view.

These different initial approaches led to get discrepancies within the fibre identification between laboratories.

Further investigations led to determine which fibre morphology attributes were the most appropriate to give sufficient clues to identify the fibre species. The suitable fibre morphology attributes are those listed in [Table 1](#). Other attributes such as the ratio lumen versus “diameter” has not been found suitable.

Moreover, the main finding was that due to the potential identification of hemp instead of flax, the involved laboratories concluded that in order to take into account all the fibre morphology attributes, both longitudinal and cross views should be carried out (except in the case of ramie is alone), as well as the application of polarized light test with the “red plate” conjunctionally the application of the twist test.

D.2 SEM longitudinal view

SEM longitudinal views of flax, hemp or ramie are similar and do not lead to give clues to differentiate these bast fibres.

D.3 “Tricky” examination

Longitudinal view (see [Figure D.1](#)) of some man-made fibres (particular polyethylene fibres) has been found to present a comparative fibre morphology attribute (referring to knots with V or X shape, see [Table 1](#)); these are “false knots” due to the spinning process (e.g. solvent evaporation, drawing). Carrying out only the longitudinal view would lead to a tricky examination, when carrying out complementary analysis could solve the situation.

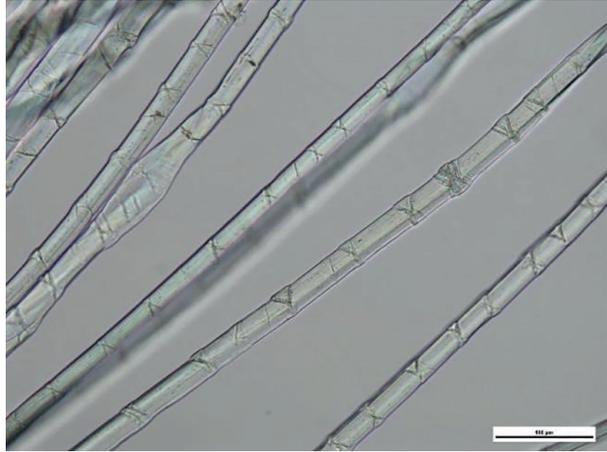


Figure D.1 — “False knots” on a man-made fibre (longitudinal view)

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Annex E (normative)

Polarized light test to distinguish flax and hemp

E.1 Rationale of the “polarised light”

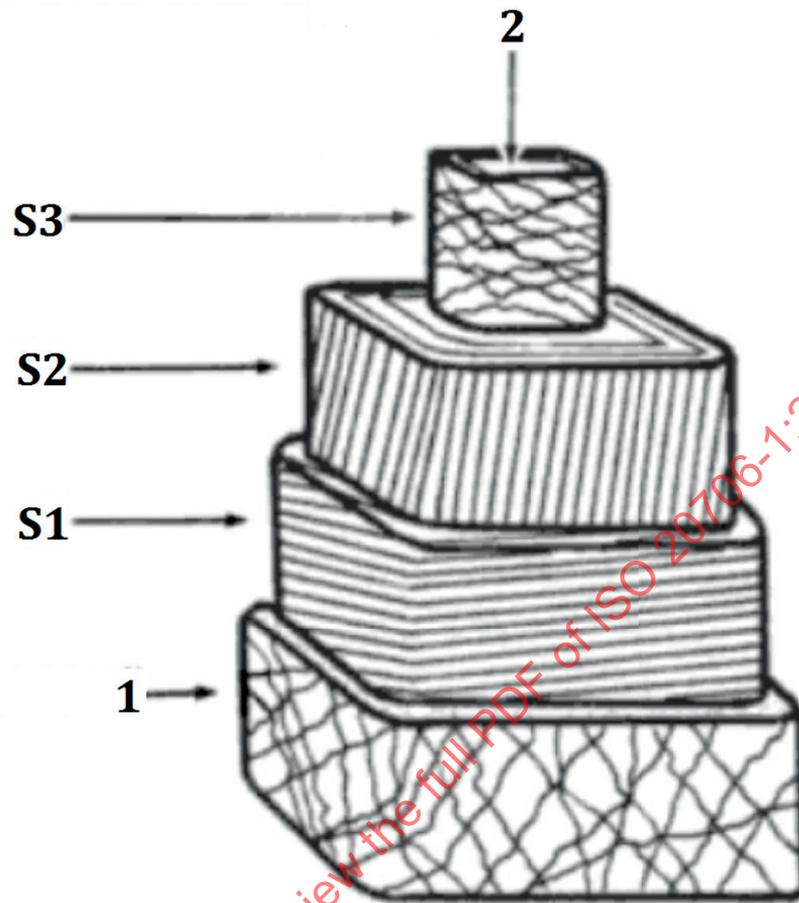
Flax and hemp have one primary cell wall and three layers of secondary cell walls.

In the primary wall, the cellulose fibrils are oriented in a random way.

The three secondary walls have specific oriented cellulose fibrils, in either the Z or S direction. When the thickness of the three secondary cell walls is nearly the same, no result can be conclusive when carried out the polarized light test. A polarized light test result can be conclusive only if one of the three secondary cell walls is significantly thicker than the two others.

Usually most of the fibres have a thicker secondary wall (see [Figure E.1](#)).

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**Key**

- | | | | |
|---|-----------------------|----|---|
| 1 | primary wall | S1 | secondary cell wall: first layer |
| 2 | cell interior (lumen) | S2 | secondary cell wall: second layer (here shown in Z-twist) |
| | | S3 | secondary cell wall: third layer |

Figure E.1 — Typical bast fibre cell showing the fibrillar orientations of the secondary cell wall layers

E.2 Principle, based on the “modified Herzog method”

In the modified Herzog test^[11], white light from the polarizer enters the birefringent specimen. With the specimen oriented at extinction (a segment of the fibre looks black), insert a 530 nm retardation plate at 45° to the crossed polars. It is claimed that this will produce additive or subtractive compensation, which causes the fibre segment to turn from extinction either slightly blue or yellow.

The colour change is said to depend on whether the fibre is S or Z-twist. A Z-twist fibre is said to turn yellow when parallel to the polarizer and blue when parallel to the analyser, while for a S-twist fibre the situation is exactly opposite.

E.3 Procedure

Set up the “Köhler illumination” which means: align field diaphragm with condenser diaphragm and set up the condenser diaphragm to about 75 % of the numerical aperture of the used objective on the light microscope.