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Table 3 is not available with this version.

Supplementary Notes S1-S3 and Tables S1-S2 are not available with this version.

Plant fibre diagnosis: refining analytical approaches using blind testing

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ABSTRACT

A range of plant fibres was used in prehistoric Europe for cordage, woven textiles and string. The analysis of degraded archaeological specimens to determine which types were being used is often challenging, partly due to uncertainties around the reliability of identification techniques. Here, two established diagnostic approaches are standardised, quantified, and blind tested in terms of their combined ability to separate reference material from nettle (*Urtica dioica* L.), flax (*Linum usitatissimum* L.) and hemp (*Cannabis sativa* L.). These approaches are longitudinal microfibrillar angle (MFA) and cross-sectional circularity (Ct) used in a two-step procedure to analyse a set of randomly selected modern fibres (n=48, 16 from each species). The overall success rate for identification was 66.7% but this varied between the species, and the results show differences in the efficacy of the methods. In the first stage, MFA distinguished between hemp and flax/nettle, with 81.3% of the hemp samples being successfully identified. Twenty-seven samples proceeded to stage 2 and were classified using Ct analysis after which 66.7% of all flax, and 50% of all nettle samples were identified successfully. This suggests that some evidence for the use of wild nettles may have been under-represented in archaeological reports.

Introduction

In understanding the details of past societies, it is essential to know which types of resources were being exploited, and how specific plants were prepared for food and other uses^{1–5}. The identification of bast fibres from archaeological textile assemblages makes an important contribution to understanding prehistoric material culture, technologies and social structures. Only a small proportion of plant products used in the past survives and establishing quality analytical methods for identifying degraded and fragmentary materials is of value to archaeological interpretation.

Current archaeological and historic fibre identification methodology relies on autoptic microscopic analytical techniques⁶. There have recently been many palaeoethnobotanical applications of fibre identification^{7–9}. However, limitations to the diagnostic methodology are occasionally noted but rarely quantified. This research programme utilises blind testing, an established method for understanding the capability and appropriateness of identification techniques^{10,11} with a broad range of examples within archaeological science^{12–20}. Since this approach is yet to be applied to bast fibre identification, understanding sources and degrees of error will help make records more robust and valuable²¹. It can also drive improvements in techniques which will aid the re-evaluation of some archaeological specimens and support discussion about the broad range of plant products in past societies.

This study aims to evaluate the viability and reliability of a combined approach using cross-sectional circularity (Ct) and microfibrillar angle (MFA) measurements as a method for distinguishing between fibres of nettle, hemp and flax. These plant fibres are considered to have been the three most commonly used bast fibres in prehistoric northern Europe. Ct is a new method presented as a quantifiable alternative to shape interpretation, commonly used as described by Suomela⁶ and evaluated by Lukesova and Holst²². MFA imaging is as presented by Bergfjord and Host²³, with the addition of directly measuring angle, and is a simpler alternative to the highly regarded modified Herzog test²⁴.

Results

All blind test results are included in Supplementary Table S2. Table 1 summarises the Ct and MFA and the diagnostic outcomes for the 48 samples. The overall accuracy of the two-step procedure is measured at 62.5%. Excluding results from specimens that would not normally be studied due to faulty sample preparation, producing very poor images, accuracy is reported at 66.7%. Accuracy for the individual species is found to be 81.3% for hemp, 50.0% for nettle, and 66.7% for flax.

Following sample preparation, one sample (retrospectively identified as flax) was considered unsuitable for MFA analysis due to an inadequate image. MFA was measured on the remaining 47 slides (Figure 1) and this feature was subsequently used to differentiate between hemp (H) and flax/nettle (FN). Eighteen samples were identified as hemp based on the positive MFA twist

angle. Only 13 of these were actually hemp, indicating that MFA, as a standalone approach, has accuracy in differentiating hemp from nettle and flax of 72%.

The 29 fibres identified as having a negative MFA, and thus likely to be flax or nettle, were subjected to a second stage of analysis by way of cross-sectional fibre circularity (Ct) was measured for each sample (Figure 2) and used here to differentiate flax from nettle. This method could not study two of the samples (both retrospectively identified as nettle).

Samples were identified as nettle if their Ct was less than 0.696 and flax if Ct was above this value. On this basis 11 samples were diagnosed as nettle and 16 as flax. Seven of the 11 'nettle' samples were correctly classified to the nettle group and 10 of the 16 'flax' samples were correctly identified to the 'flax' group.

Discussion

The 66.7% overall accuracy achieved in this blind test appraisal offers the first quantitative data to illustrate the capability of MFA and Ct within a two stage diagnostic methodology, frequently used methods for differentiating between three common bast fibres. The result is 62.5% if only the samples that could be fully studied are included in the calculations as, in application those poor samples may be replaced by a further fibre from the archaeological material. In an applied scenario the three excluded samples would be described as indeterminate. Deciding not to decide²⁵ is an important step in this process. Considering the results from the different plant species within the test it is clear that nettle fibres are the most likely to be misidentified (58% of all nettle sample submitted were correctly identified) and nettle fibres are the most likely to be rejected as bad samples. It is consequently suggested that nettle use in the past may be under-represented, not only because it may be overlooked as a useful fibre but also by the nature of the analytical processes leading to its identification in archaeological and historic material.

The results show that the method has performed relatively well within the test scenario. Random judgement would result in an accuracy of around 33%. The most decisive element of the method is stage 1, MFA analysis. 81% of hemp samples were correctly identified at this stage. A review of the data finds that five samples (two nettle and three flax) were misidentified as having a positive MFA value. Revisiting the samples has confirmed that it is not that these samples have a positive MFA but rather the that adequate microscope imagery is sometimes difficult to achieve and results in misreading imagery misinterpretation. This is one of the typical challenges faced by subjective procedures, and variation is likely to be seen in the quality of MFA measurement depending on analyst experience and sample quality. Beyond a test scenario there is also the possibility for cognitive bias to influence the determination. It is widely understood that external influence can impact on the analytical capability of a specialist, see²⁶ for an example. Therefore it is recommended that analysts attempt to shield themselves from external influence before conducting analysis, as proposed elsewhere²⁷. Given the high possibility of this type of error, finding a replacement for the MFA procedure outlined here should be a high priority for research.

In the evaluation, the Ct for hemp samples that erroneously proceeded to the second step is excluded, since their data points add little value to the quality of the Ct analysis that separates nettle and flax. In the second stage of analysis 24 nettle and hemp samples were studied, 17 of which were correctly identified. This places the accuracy of Ct analysis at 70.8%. Error in diagnosis here is influenced by overlaps in fibre form between the two taxa. The presented methodology has caveats, with implications for using the method in wider applications. The cut-off value of Ct 0.696 to distinguish flax from nettle is based on a relatively limited dataset. Cross-sectional circularity data and the overlap between flax and nettle may vary much more than has been established to date. There is a need for a larger reference collection to be evaluated for MFA and Ct in order to refine the criteria used in diagnosing unknown samples.

Additional limitations relate to archaeological preservation and sample processing methods. In selecting samples to be subjected to the blind test, it was decided to concentrate on fibres that were minimally processed. Processing by combing may have removed more damaged bast fibres or damaged the ones that remain, and allowed for an easier sample selection and microscopic imaging. However, given that only three samples (6%) could not be studied due to the poor condition of the image this may not be a substantive issue. More significant is the effect of degradation on the quality of the fibres from archaeological deposits and therefore the ability to use the presented methods. Further research is needed to understand how robust the presented methodology is when applied to samples that have undergone a range of degradation processes in various burial environments. Despite the acknowledged caveats the results of this paper mark a step forward in evaluating the integrity of fibre identification methods as quantitative protocols improve the understanding of identification error rates and improve repeatability.

Methods

Selection of samples

Sixteen samples of each bast fibre, nettle, flax and hemp, were randomly selected and individually bagged in private by AAE and presented as blind samples to DBW for analysis. Each sample typically included up to four technical fibres selected from

each species' fibre collection. Commercial suppliers were not used as errors in selection have been known²⁸, therefore the fibre samples used were harvested locally (see table 2).

MFA sample preparation and procedure

Following²⁹, samples of fibre, circa 2mm in length, were cut with a scalpel. These were separated by hand, mounted on a slide in distilled water under a glass coverslip, and sealed with clear lacquer.

Samples were viewed in transmitted light under a Nikon Optiphot compound microscope, at 100x and 400x magnification, with cross-polarised lighting and a full wave plate when necessary to enhance contrast. The MFA for each fibre was determined from the angle of variance as measured twice, first, by direct angular variations indicated on the rotating stage scale and secondly, using Olympus AnalySIS software to measure the enclosed angle. The mean of these two angular measurements was used as the determinant value for classification purposes. When viewing the fibre, focusing on the anterior surface is essential, as the posterior view is also visible with translucent fibres, and microfibre angles are displayed at the opposite angle.

It is well established that the angular orientation of fibres within the stems of nettle and flax have a twist that is opposite to that of hemp^{23,24}. As a result, the measurement, and notation of a positive or negative angle, is usually considered a robust technique for distinguishing hemp from nettle/flax.

Cross-sectional circularity (Ct) sample preparation and procedure

A scalpel was used to cut 5mm lengths of each technical fibre, circa 15-25 μ m diameter. These were centralised vertically within a 6mm diameter mounting tube sealed at the lower end. Alongside the fibre was placed a 250 μ m copper wire (which would serve as a trace later in the procedure). EpoFix (Struers (UK) Ltd. 2016) low viscosity epoxy resin was used to fill the tube and left to set. Following overnight curing, the moulded sections were ground and polished prior to microtome cutting of 1mm thick CSA samples suitable for microscope imaging. The CSA samples were mounted on glass slides with Eukitt mounting medium.

A Nikon Optiphot compound microscope with BD Plan 40x 0.65NA objective lens was used to image the cross-sections of each technical fibre bundle in reflected light. The multi-fibre CSA images were evaluated using ImageJ software³⁰. The groups of individual fibre cross sectional outlines were manually traced within the image on the screen; the programme analyses, displays, and edits the image formats to calculate area, circularity, and shape ratios.

Six representative fibre images were randomly selected from each technical fibre group. The samples were subsequently analysed in ImageJ software to record the circularity (Ct) ratios. A database of the measured fibre circularity for the 48 blind test samples is included in Table 3.

Full details of both methods are described in supplementary note S1

Fibres were assigned to either flax or nettle based on Ct value. If the Ct value was less than 0.696, it was determined to be nettle, while if it was above than this value it was determined to be flax. The rationale for using this cut-off value is described in supplementary note S3.

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Author contributions statement

A.A.E, G.B.T, and D.B.W conceived the experiment. D.B.W provided samples for analysis, and carried out the experiment as the blind analyst. A.A.E conducted the experiment, prepared the blind test samples and reviewed the results. All authors collaborated on analysis and reviewed the manuscript.

Additional information

Datasets

Images that form the raw dataset produced during the blind test can be found at <http://doi.org/10.5281/zenodo.3384962>³¹

Competing interests

The authors declare no competing interests.

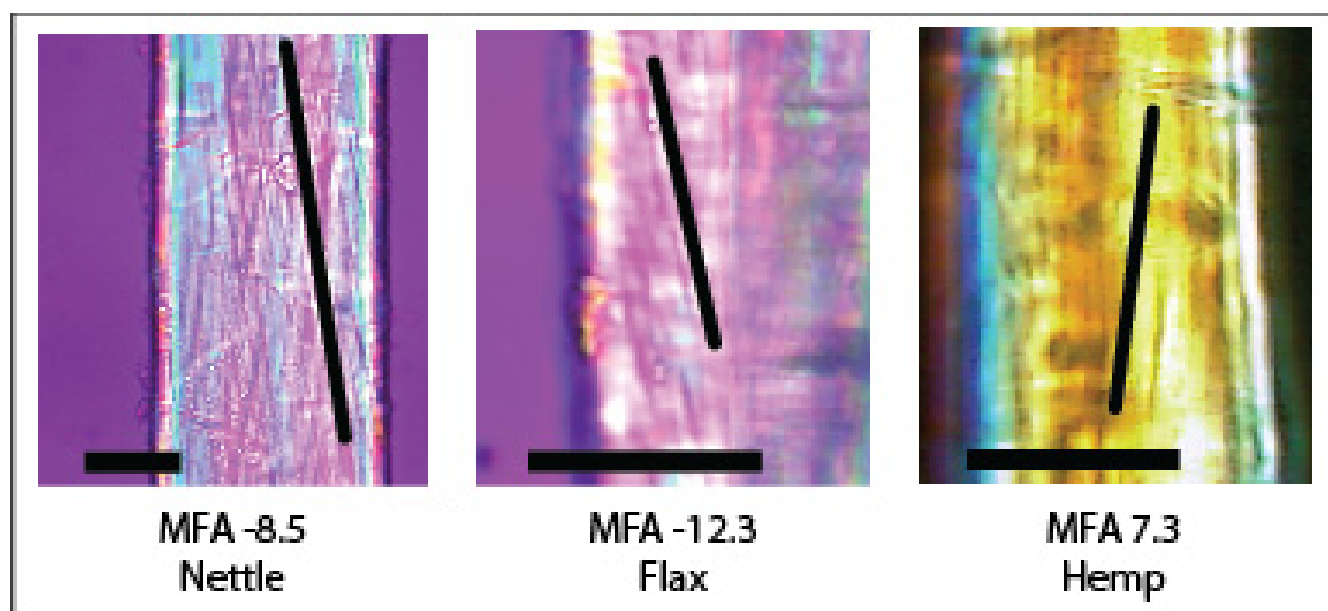


Figure 1. Examples of the three different fibres in the study and the measurement of MFA by direct observation and digital angle measurement. Scale bar represents 25 micrometers

	Nettle	Flax	Hemp	
Samples selected and prepared	16	16	16	
Samples studied for MFA (stage 1)	47			1 rejected
Grouping based on MFA	29		18	
Samples studied for Ct (stage 2)	27		–	2 rejected
Grouping based on MFA and Ct	13	14	18	
Number correctly identified	9	10	13	
Total accuracy	56.3%	62.5%	81.3%	66.7
Accuracy without rejects	64.3%	66.7%	81.3%	71.1%

Table 1. Summary table of Blind test results

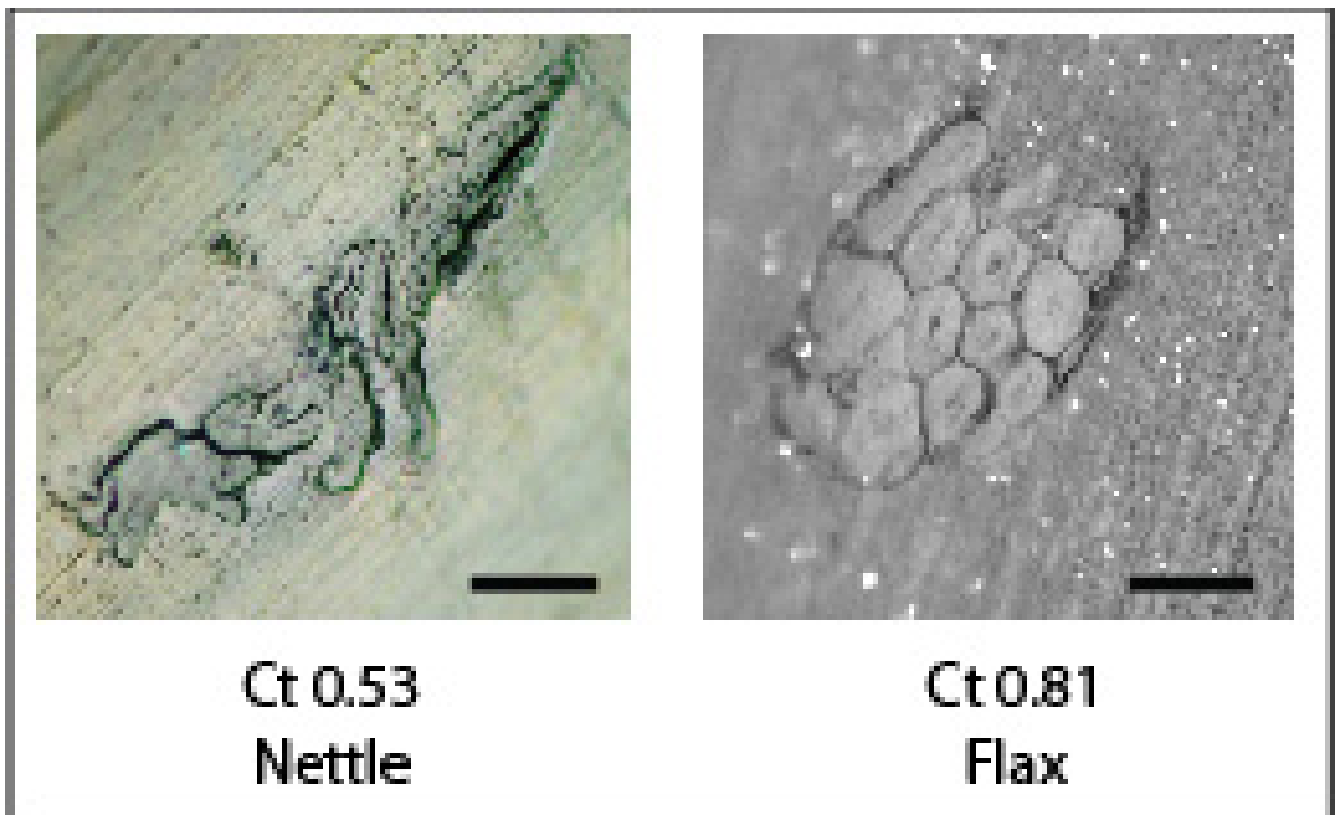


Figure 2. Examples of Nettle and Flax fibres and differences in cross-sectional appearance and Ct measurement. Scale bar is 25 micrometers

Fibre	Source	Location	Processing
Nettle	Testbed crop	Bradford, UK	Retted, manual decortication, basic combing
Hemp	Commercial crop	Baswick, UK	Retted, mechanical decortication, basic combing
Flax	Testbed crop	Bradford, UK	Retted, manual decortication, basic combing

Table 2. Source of blind test plant fibre samples