

Polarised light microscopy: an old technique casts new light on Māori textile plants

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ABSTRACT

Understanding the composition of an artefact has ramifications for advancing human history and behaviour knowledge, providing cultural information about trade, agricultural practices and adaptation to new environments. However, accurate plant identification from artefacts is problematic, since textile production, age, dirt and/or conservation treatments obscure morphological features, and specimen size and/or ethical considerations hamper modern analytical methods. This study tested the efficacy of polarised light microscopy (PLM) in the identification of New Zealand plant species commonly used in Māori textiles, and demonstrates that morphological and birefringent features observed using PLM have the potential to distinguish between- and within- plant genera.

KEYWORDS

Māori textiles, New Zealand flax, *Phormium*, *Cordyline*, *Freycinetia*, sign of elongation, modified Herzog test, plant material identification

INTRODUCTION

Accurate plant material identification is critical for advancing study of material culture, since an object's composition provides an insight into its origin, additionally revealing important cultural information such as human interactions and emigration pathways (Schaffer 1981; Jakes *et al.* 1994). However, one of the main challenges for accurate identification of plant species in textile artefacts is the scarcity of distinct morphological features, evident from whole plants or individual leaves (e.g. leaf arrangement and shape), that remain once the plant is processed into a textile material such as fibre or leaf strips for weaving. Species-specific morphological characteristics of animal hair (e.g. medulla shape and size, cuticular scale pattern, Tridico 2009) often facilitate, if present, the identification of animal fibres from artefacts, although processing and ageing can limit identification to higher taxonomic levels (Tridico *et al.* 2014). Processing and age of plant materials can likewise pose ongoing challenges for textile scientists, archaeologists, conservators and museums professionals, since processing of plant material for textile production frequently obscures macroscopic diagnostic features (Jakes *et al.* 1994), and even microscopic features of different plant species can appear remarkably similar (Bergfjord *et al.* 2010; Haugan and Holst 2014).

Recent research has highlighted the ramifications of incorrect plant identification in European textile artefacts (e.g. Haugan and Holst 2014), with the misidentification of fibres from European flax (*Linum* spp.) considered to have hampered understanding of the nexus between textile plant cultivation and the development of agriculture (Bergfjord *et al.* 2012). However, such misidentification of plant fibre species, and the potential misrepresentation of the history of particular plant species, is not only restricted to artefacts of European origin. For example, the majority of Māori textiles from New Zealand, represented extensively in national (e.g.

Museum of New Zealand Te Papa Tongarewa) and international collections (e.g. British Museum, London, Swedish Museum of Ethnography, Stockholm), are generally assumed to be constructed from harakeke (*Phormium tenax*, New Zealand flax). This is despite a range of other indigenous New Zealand plant species being recognised as important in the production of Māori textiles (e.g. tī tōi *Cordyline indivisa*, tikumu *Celmisia* sp. and houi *Hoheria* sp. for kākahu/cloaks, tī kōuka *Cordyline australis* for pāraerae/sandals, kiekie *Freycinetia banksii* for kete/baskets; McCallum and Carr 2012; Tamarapa and Wallace 2013).

In the case of Māori textiles, plant materials are often identified as harakeke, based on a very limited, and mostly macroscopic, range of characteristics thought to be ‘typical’ for this species (e.g. leaf material – shiny epidermis, even veins and coloured midrib and margin (if present); fibre - soft and pliable with very fine, round and lustrous individual strands; Goulding 1971). These external morphological features may be lost due to degradation or when a leaf’s fibre component is extracted to obtain muka/fibre aggregates (fibre bundles) used in the production of fine woven garments (e.g. korowai or kākahu / cloaks). Accurate plant material identification from internal anatomical features also remains difficult for objects comprised of leaf strip as the removal of a specimen necessary for preparing transverse sections for identification of morphological features may be considered ethically or culturally unacceptable, and still may not result in a definitive diagnosis (Smith *et al.* 2014). Thus recent research efforts have focussed on developing less invasive identification techniques that use either fragments that have become detached from artefacts or minute samples removed directly from an artefact. Such samples are still sufficient to allow for diagnostic measurements at the cellular level, for example microscopic characteristics of individual harakeke fibre (ultimate) cells have been summarised (e.g. cell length, width; Carr and Cruthers 2007; Carr *et al.* 2008). However, a lack of knowledge concerning the

magnitude of intra- specific variation and inter-specific overlap in these characteristics amongst plant species known to be used for Māori textiles production diminishes the potential identification power of even precise microscopic measurements. Similar problems with plant species misidentification is noted in European artefacts, where the narrow range of microscopic procedures applied, coupled with reliance on microscopic features long accepted as being typical (rather than diagnostic) of particular plant species (Haugan and Holst 2014), makes some previous positive identification of aged plant material questionable.

Textile plant species identification using microscopy requires maceration of a small sample of the textile in question (e.g. a fragment of fibre, processed leaf or stem) to release individual fibre cells for observation (The Textile Institute 1975). The basic structure and range of morphological features typical of plant fibre cells are well recognised (Eder and Burgert 2010; Gibson 2012), with Preston's (1974) microscopic observations of cell structure still the most thorough and insightful treatment. Variations in the basic fibre cell structure and features may be due to a number of factors including; taxonomic grouping, genotype, position within the plant (root, stem, leaf, fruit), age of the fibrous tissue, maturity of the individual cell, the growing conditions of the plant and the processing route used to transform the plant into a textile material (Preston 1974; Eder and Burgert 2010; Long *et al.* 2010). The relative importance of these factors in determining final fibre cell dimensions is poorly understood. Despite this, the major microscopic features of fibre cells from common industrial textile plant species are considered well characterised (Luniak 1953; McCrone and Delly 1973; Catling and Grayson 1982).

In an attempt to improve fibre identification from woven artefacts, a plethora of modern analytical techniques have been utilised including DNA (Hofreiter *et al.* 2001), micro-

computed tomography (μ CT; Smith *et al.* 2013) and scanning electron microscopy (SEM; Cartwright 2013). However, access to specialised, and at times expensive equipment, and/or the professional training required to apply these techniques often restricts their application to a small percentage of cultural institutions with sufficient funding dedicated to such purposes. Furthermore, in many instances, modern analytical techniques have proven limited in identifying the plant species comprising artefacts of interest (e.g. μ CT identified 50% artefacts to species level, Smith *et al.* 2013; unidentifiable plant fibres by SEM from Cook Voyage Collections, Cartwright 2013). More recently, researchers have attained considerable success in plant species identification from individual fibre cells by revisiting a relatively inexpensive technique, polarised light microscopy (e.g. Bergfjord and Holst 2010; Skoglund *et al.* 2013). Compared with standard light microscopy, polarised light microscopy (PLM) enables a greater range of fibre cell features and properties to be observed (e.g. cross-markings, dislocations, crystals), due to the enhanced contrast possible with polarised light and the optical behaviour of the fibre cells when viewed with crossed polars and wavelength specific filters (wave plates or compensators, Preston 1933; Luniak 1953; McCrone 1999; Palenik 1999). Systematic observation under polarised light of cell morphology, associated crystal morphology and cell birefringent properties (Herzog 1955; Ilvessalo-Pfäffli 1995; Haugan and Holst 2013) may enable recognition of a suite of features typical of fibre cells of a given plant species (Bergfjord and Holst 2010; Haugan and Holst 2014).

Whilst recent research has highlighted the discriminatory power of PLM techniques to identify plant fibres present in European artefacts, the technique had only been applied to bast fibres from dicot species belonging to taxonomically distinct families or genera (e.g. family, genus; flax: Linaceae, *Linum*; nettle: Urticaceae, *Urtica*; hemp: Cannabaceae, *Cannabis*); Bergfjord and Holst 2010; Bergfjord *et al.* 2012; Haugan and Holst 2014). In contrast,

indigenous textile plants in New Zealand (and the Pacific) are almost all monocots, with leaf, rather than bast material the main plant part used for textiles. Similarities between leaf and bast fibre cells in their function (mechanical support and protection of vascular tissue) and cell wall structure (a thick secondary cell wall consisting of multiple layers; cellulose microfibrils within each layer aligned in a spiral to the longitudinal cell axis; Eder and Burgert 2010) indicate that PLM techniques may also provide a means to discriminate among New Zealand monocot species. However the latter are a much less diverse group in terms of leaf morphology, and often belong to a single genus (e.g. *Cordyline* cabbage trees (Asparagaceae): *C. australis*, *C. indivisa*, *C. banksia*); no information is currently available as to whether PLM techniques would still provide species-specific diagnostic characteristics under these more constrained parameters. To address this significant gap in knowledge, this study systematically investigated the morphological and birefringent characteristics of fibre cells extracted from plant taxa recognised as key fibre and leaf material for Māori textiles, in order to evaluate the potential for PLM techniques to discriminate among leaf fibres of culturally important, closely related plants.

MATERIALS AND METHODS

Sample collection

Samples were obtained using customary harvesting and fibre extraction techniques (see Te Kanawa *et al.* 2006 for details; McCallum and Carr 2012) from five New Zealand monocot species recognised as key Māori textile plants (e.g. Pendergrast 1996) and through consultation with expert weavers (R. Ngarimu-Cameron *pers. obs.*); harakeke *P. tenax* (Hemerocallidaceae), tī kōuka *Cordyline australis* cabbage tree, tī ngahere *Cordyline banksia*

forest cabbage tree, tōi *Cordyline indivisa* mountain cabbage tree (Lomandroideae), kiekie *Freycinetia banksii* (Pandanaceae; Table 1). A single leaf was harvested from three individual plants of each species, with the exception of tī ngahere, where three leaves were obtained from a single plant. Multiple cultivars (Harris *et al.* 2005; Scheele 2005) and/or phenotypes (i.e. plants with observable sets of physical characteristics) of harakeke are recognised based on both traditional knowledge and morphological characteristics (Scheele 2005). Our study included harakeke specimens collected from a number of bushes valued by weavers (one named cultivar and three un-named phenotypes) to assess the potential variability in harakeke fibre cell characteristics. The potential influence of provenance and growing locality on ultimate fibre cell characteristics was also assessed for harakeke and kiekie by sampling from multiple harvest locations (Table 1). Fibre aggregates were extracted by removing the margin and mid-rib from each leaf prior to removal of the epidermal layer from the upper surface, or in the case of kiekie and tī species, soaking for an extended period of time in water prior to extracting fibre aggregates (retting, Goulding 1971; Te Kanawa *et al.* 2006). Single leaf samples were also obtained from two sub-species of wharariki/mountain flax, *Phormium cookianum* subspecies *cookianum* and *P. cookianum* subspecies *hookeri*. Although wharariki is considered less common than harakeke in the production of woven objects (Goulding 1971), weavers were likely to use locally abundant fibre plants if preferred species were unavailable and thus species may be present in a number of museum objects previously assumed to be made of harakeke. To reflect the tendency for wharariki to be used as leaf strips rather than fibre bundles, fibre aggregates were not extracted. All samples were dried at ambient temperature (18°C) and in natural diffuse light before being stored in archival acid-free paper bags until further processing.

Sample processing

To obtain ultimate fibre cells from each specimen, 10 mm long sections of fibre aggregates or 10 mm x 5mm leaf samples were cut from larger samples and placed into individual 1.5 ml Eppendorf tubes containing approximately 0.8ml of 1.2% w/v sodium hypochlorite. Each capped tube was placed into a 60°C water bath for two hours, then samples were rinsed with distilled water. Each sample was then transferred into a new Eppendorf tube containing 0.8ml of distilled water and vigorously shaken (10-20 seconds) to separate the ultimate fibre cells. Uncapped tubes were placed in a 40°C drying oven for 48 hours, then stored at ambient conditions (18°C) prior to observations using microscopy.

Polarised light microscopy

Five sub-samples of ultimate fibre cells were mounted on glass slides in distilled water (with cover slips) with a minimum of 10 ultimate fibre cells examined per slide from each individual leaf/plant/species (Table 1). Fibres were examined under polarised light using a Olympus System Microscope Model BX41 fitted with a rotating stage and optional full wave compensator of wavelength 525nm. An integrated Canon EOS 1100D camera captured images using AxioVision software (Version 4.8.2.0 Carl Zeiss MicroImaging GmbH).

For each species, the fibrillar orientation was first observed (i.e. S- or Z-twist) using the modified Herzog test (red plate test, Haugan and Holst 2013). Secondly, we quantified whether fibres attained full or partial extinction under crossed polars (ie. invisible or partially visible fibre in relation to the black background) when fibres were aligned to the plane of polarised light ($\alpha = 0^\circ$ and 90°), with Haugan and Holst (2013) suggesting that partial fibre

extinction reduces the ability of the modified Herzog test to determine the fibrillar orientation. Thirdly, we assessed the sign of elongation (SE) by examining the fibre colour at $\alpha = 45^\circ$ and 135° , with a positive SE considered characteristic of most textile fibres (parallel $n_{\parallel} >$ perpendicular n_{\perp} refractive index, Luniak 1953; Wheeler and Wilson 2008). For both the SE and modified Herzog tests, results were classified as 'indeterminate' when inconsistent colour changes were observed.

Morphological characteristics of ultimate fibres were described using features illustrated by Ilvessalo-Pfäffli (1995). In particular, the general fibre cell shape, surface markings (e.g. cross-markings, dislocations), lumen shape, fibre cell end shape (e.g. pointed, blunt, scimitar-like), fibre cell wall irregularities (e.g. scalloping, pits) and associated crystals (e.g. cuboidal or elongated styloids, raphides, Prychid and Rudall 1999) were described. The relative abundance of cell pits were also classified as low (Figure 1a-b), moderate (Figure 1c-d) or high (Figure 1e-f). Fibre cell dimensions (length mm, width μm) for each species as described by Carr and Cruthers (2005-2007) were reported, with the exception of *P. cookianum* subspecies *hookeri* for which dimensions were assessed from 10 individual cells.

Birefringence was assessed by mounting groups of fibre cells from each plant species in a series of Cargille™ refractive index oils (Series A, Range nD 1.460-1.640, adjustment ± 0.0002 ; $20 \pm 2^\circ\text{C}$). The Becke line test (Becke 1893; Preston 1947) was used to determine n_{\parallel} and n_{\perp} refractive indices, from which the birefringence (Δn ; $n_{\parallel} - n_{\perp}$) and the isotropic refractive index (n_{iso} ; $\frac{1}{3}(n_{\parallel} + 2n_{\perp})$) was calculated. Additionally, differences in n_{\parallel} and n_{\perp} refractive indices between fibre plant species of New Zealand and European origin were represented graphically (described from Luniak 1953).

Deposition of voucher specimens

Permanent reference slides of contemporary specimens prepared using Cargille Meltmount™ QUICK STICK (nD = 1.662) were deposited at Department of Applied Sciences - Clothing and Textiles, University of Otago, New Zealand; Otago Museum, New Zealand; Te Papa Tongawera National Museum of New Zealand.

RESULTS

General remarks

With the exception of kiekie, identification of fibre cells required multiple morphological and optical characteristics to be quantified due to similarities among multiple species. For example, fibrillar orientation (via modified Herzog test) was either Z-twist (5/7 species) or indeterminate (2/7 species; tī tōī, kiekie), and a positive SE was observed for five out of seven species, with the SE difficult to determine for tī tōī and the harakeke cultivar Makaweroa. There was no observable relationship between the ease with which the fibrillar orientation was determined and the degree of extinction when fibres were aligned to the plane of polarised light. Pitted cell wall regions of kiekie and *Cordyline* species remained visible even when the remainder of the fibre cell reached extinction.

Kiekie was easily distinguished from all other species by a single distinctive characteristic, the presence of abundant cuboidal styloid crystals (Figure 2a), a feature which remained consistent despite differences in provenance and growing location of the observed specimens. Whilst cuboidal styloid crystals were only observed from kiekie specimens, other crystal

shapes were noted from *Cordyline* species tī kōuka (elongated styloid, conglomerate; Figure 2b) and tī ngahere (raphide; Figure 2c), and from harakeke Phenotypes 2-3. Crystals were not observed associated with ultimate cells of tī tōī, wharariki subspecies or the harakeke cultivar Makaweroa investigated in this study.

The general fibre cell morphology was similar among harakeke specimens, wharariki subspecies and tī ngahere, all of which tended to have long and slender cells with cross-markings and dislocations (cross-markings/dislocations Figure 3); however the presence of moderately abundant diagonal elongated pits on tī ngahere cells differentiated tī ngahere from *Phormium* species (Figures 1c, 3d). Furthermore, the presence of cell wall swelling associated with dislocations on wharariki subspecies was useful for differentiating between *Phormium* species (Figure 3b). Elongated, diagonal cell wall pits were characteristic of all *Cordyline* species and kiekie, with the abundance of pits ranging from moderate abundance in tī kōuka and tī ngahere (Figure 1c-d) to high abundance in tī tōī and kiekie (Figure 1e-f). Pits were rare or absent from all harakeke cultivars/phenotypes and wharariki subspecies examined.

Birefringence was highly variable for each New Zealand species; with a high degree of overlap in both n_{\parallel} and n_{\perp} refractive index variance (Figure 4). However, all New Zealand species had a considerably lower n_{\parallel} refractive index in comparison to values for European fibre species reported in the literature (which are highly variable).

Fibre cell features

Specific results for each plant species examined in this study are provided below.

Harakeke *Phormium tenax* New Zealand flax J.R. Forst & G. Forst

Figure S1a-d

Z-twist; indeterminate (Makaweroa) or positive SE (Phenotype 1-3); partial extinction at crossed-polars. Long, cylindrical fibre cells with even, narrow lumens (less than 1/3 cell width). Pointed fibre ends. Cross-markings frequent. Dislocations present and not associated with swelling of cell wall. Fibre cells often broken into smaller fragments. Pits absent or rare. Raphide crystals may be present (Phenotype 2-3). Length (mean \pm sd) 1.63 mm \pm 0.3 mm - 4.27 mm \pm 0.7 mm, width 11 μ m \pm 2 μ m - 15 μ m \pm 2 μ m (Carr and Cruthers 2005-2007). Refractive index (mean \pm variance, n = 3): n_{\parallel} 1.552 \pm 0.017, n_{\perp} 1.515 \pm 0.002 (Figure 4). Birefringence (Δn) 0.037 \pm 0.015. Isotropic refractive index (n_{iso}) 1.527 \pm 0.007.

Wharariki *Phormium cookianum* subspecies *hookeri* coastal flax

Figure S2a-e

Z-twist; positive SE; partial extinction at cross-polars. Long, cylindrical fibre cells with narrow lumens (less than 1/3 cell width). Outer cell walls may be uneven. Pointed ends. Cross-markings frequent. Dislocations present and not associated with swelling of cell wall. Fibre cells often broken into smaller fragments. Pits absent or rare. Crystals absent. Length (mean \pm sd) 3.29 mm \pm 0.96 mm; width 17 μ m \pm 2 μ m. Refractive indices not assessed.

Wharariki *Phormium cookianum* subspecies *cookianum* mountain flax

Figure S3a-d

Z-twist; positive SE; partial extinction at cross-polars. Long, cylindrical fibre cells with narrow lumens (less than 1/3 cell width). Outer cell walls may be uneven. Pointed ends. Cross-markings frequent. Dislocations associated with swelling of cell wall. Fibre cells often broken into smaller fragments. Pits absent or rare. Crystals absent. Length (mean \pm sd) 3.95 mm \pm 0.51 mm; width 13 μ m \pm 1 μ m (2WhWhiB, Carr and Cruthers 2005-2007). Refractive

index (single sample): n_{\parallel} 1.561, n_{\perp} 1.519 (Figure 4). Birefringence (Δn) 0.042, Isotropic refractive index (n_{iso}) 1.533.

Tī kōuka *Cordyline australis* cabbage tree (Forst. f.) Endl.

Figure S4a-c

Z-twist; positive SE; partial extinction at cross-polars. Smooth, cylindrical fibre cells, though scalloped cell walls occasionally occur. Lumen width consistent within single cells, but variable among cells. Blunt cell ends. No dislocations or cross-markings. Moderate abundant diagonally orientated elongated pits (fewer than tī tōī). Elongated styloid or conglomerate crystals may be present. Variable cell length, greater cell width than tī ngahere. Length (mean \pm sd) 1.01 mm \pm 0.3 mm - 1.39 mm \pm 0.52 mm; width 13 μ m \pm 3 μ m (Carr and Cruthers 2005-2007). Refractive index (mean \pm variance, n = 3): n_{\parallel} 1.552 \pm 0.009, n_{\perp} 1.517 \pm 0.006 (Figure 4). Birefringence (Δn) 0.034 \pm 0.009. Isotropic refractive index (n_{iso}) 1.529 \pm 0.006.

Tī ngahere *Cordyline banksii* forest cabbage tree Hook.f.

Figure S5a-d

Z-twist, though may be difficult to determine; positive SE; full extinction at cross-polars. Long, smooth, cylindrical fibre cells with lumen \sim 1/3 of cell width. Fibre cell ends may be pointed, blunt or scimitar-like. Cross-markings and dislocations present (less abundant than harakeke). Moderately abundant diagonally orientated pits (fewer than tī tōī). Raphide and/or elongated styloid crystals may be present. Length (mean \pm sd) 1.44 mm \pm 0.49 mm; width not measured (Carr and Cruthers 2005-2007). Refractive index (mean \pm variance, n = 3): n_{\parallel} 1.558 \pm 0.013, n_{\perp} 1.516 \pm 0.009 (Figure 4). Birefringence (Δn) 0.041 \pm 0.003. Isotropic refractive index (n_{iso}) 1.530 \pm 0.010.

Tī tōī *Cordyline indivisa* mountain cabbage tree (G.Forst.) Steud.

Figure S6a-e

Twist direction difficult to determine; positive, negative or indeterminate SE may be observed; full extinction at cross-polars except for pits. Cylindrical fibre cells with wide lumens ($>1/3$ cell width). Blunt cell ends. Cell walls can be lightly scalloped. Cross-markings and dislocations absent. Highly abundant diagonally orientated, elongated pits. Crystals absent. Variable cell length, greater cell width than other *Cordyline* species. Length (mean \pm sd) 1.15 mm \pm 0.2 mm - 1.45 mm \pm 0.3 mm; width 15 μ m \pm 2 μ m (Carr and Cruthers 2005-2007). Refractive index (mean \pm variance, n = 3): n_{\parallel} 1.533 \pm 0.014, n_{\perp} 1.522 \pm 0.005 (Figure 4). Birefringence (Δn) 0.011 \pm 0.009. Isotropic refractive index (n_{iso}) 1.525 \pm 0.008.

Kiekie *Freycinetia banksii* A.Cunn

Figure S7a-d

Twist direction difficult to determine; positive SE; full extinction at cross-polars except for pits. Blunt cell ends. Wide lumens ($>1/2$ cell width). Cross-marking absent. Abundant pits. Abundant cuboidal styloid crystals. Outer cell wall may show scalloping in association with evenly spaced cuboidal styloid crystals. Length (mean \pm sd) 0.68 mm \pm 0.16 mm; width 13 μ m \pm 3 μ m - 18 μ m \pm 3 μ m (Carr and Cruthers 2005-2007). Refractive index (mean \pm variance, n = 3): n_{\parallel} 1.547 \pm 0.006, n_{\perp} 1.526 \pm 0.005 (Figure 4). Birefringence (Δn) 0.022 \pm 0.001. Isotropic refractive index (n_{iso}) 1.533 \pm 0.005.

DISCUSSION

This study has demonstrated that morphological and birefringent characteristics of fibre cells observed using PLM techniques are highly useful in discriminating among leaf fibres of indigenous New Zealand monocot plant species commonly used to produce Māori textiles. The ubiquitous presence of cuboidal styloid crystals proved the single most useful diagnostic feature for identifying kiekie specimens, which shared many other morphological features

with *Cordyline* species tī kōuka and tī tōī (e.g. cell shape, pits). Preliminary studies by C. Smith et al. (unpublished) and J. Davies (unpublished honours thesis) also suggest that cuboidal styloid crystal presence in kiekie is not influenced by specimen age (+150 years) nor provenance, thus reinforcing the capability of this feature to correctly identify kiekie specimens. Whilst other crystal shapes were observed from tī kōuka and tī ngahere (*Cordyline* species), there was great variability as to whether crystals would be observed when ~ 100-500 fibre cells were examined, thus the presence or absence of crystals has a low capacity for differentiating among other plant species. Furthermore, our results support previous findings that crystal presence in harakeke may be cultivar- and phenotype-specific (e.g. Waihirere cultivar, Karikari Beach phenotype, Carr and Cruthers 2005-2007; Carr and Cruthers 2007), however the presence of raphide crystals in harakeke Phenotypes 2-3 (unknown cultivars, Table 1) is in direct contrast to Prychid and Rudall (1999) findings, which noted an absence of raphide and presence of styloid crystals in *Phormium* species.

Both the SE and fibrillar orientation were shown to have limited power to differentiate among New Zealand plant species, with most demonstrating both a positive SE and Z-twist. However, the fibrillary orientation was consistently difficult to quantify from both highly pitted tī tōī and kiekie fibre cells, whereas a Z-twist was clearly evident from other less pitted *Cordyline* species, tī kōuka and tī ngahere. The ability to determine the SE of harakeke was highly dependent on cultivar/provenance (positive: harakeke Phenotypes 1-3; indeterminate: Makaweroa), thus both measures have poor classification potential to differentiate *Phormium* species. Despite this, a number of fibre cell characteristics of harakeke differentiate this species from the fibre cells of wharariki and tī ngahere, the two species most likely to be confused with harakeke. In particular, harakeke's long, thin fibres, with absence/rare pits (abundance per cell; harakeke <2 vs. 20+ tī ngahere), frequent cross-markings and

dislocations not associated with enlarged cell walls (a characteristic of wharariki), enable this species to be easily differentiated from otherwise similar species used in woven objects. Our study also demonstrates opposing fibrillar orientation between harakeke (New Zealand flax; Z-twist if determined) to that of otherwise morphologically similar (in terms of cell shape, cross-markings and dislocations) European flax (*Linum* sp., S-twist; Haugan and Holst 2014). Although determination of fibrillar orientation using the modified Herzog test has been mostly applied to bast fibres, the technique is potentially applicable to any fibre cell having microfibrils arranged in a helical structure within the cell wall (Valaskovic 1991), including leaf fibres (e.g. sisal and abaca; Petraco and Kubic 2004). However, the clarity of results obtained using this technique is dependent on the secondary cell wall structural characteristics, such as the number and thickness of cell wall layers (Valaskovic 1991), and may be further complicated by localised variations in microfibril angle associated with pits or dislocations (Eder and Burgert 2010). Investigations of palm and bamboo species indicate monocot fibre cells may have more secondary cell wall layers than bast fibre cells (Liese 1987, Tomlinson 2006), although pineapple leaf fibre cells have been reported as having only three layers (Wan Nadirah *et al.* 2012). Such variability in cell wall layering, if present in New Zealand monocot fibre cells, may have contributed to the difficulty in determining the fibrillar orientation of some species assessed here. However, at present the fine detail of secondary cell wall structure of New Zealand fibre species remains unknown.

The usefulness of birefringence measures for distinguishing among New Zealand plant species was also low due to significant overlap in both n_{\parallel} and n_{\perp} refractive indices when intraspecific variation was accounted for. Such variation in refractive indices is rarely acknowledged in fibre identification literature, with a tendency to report mean values only (e.g. Wheeler and Wilson 2008; Goodman 2009; but see Luniak 1953). This incorrectly

suggests that a high degree of precision, and therefore discriminatory function, can be achieved from assessment of refractive index (although the need to sample adequately, condition fibres, control temperature and relative humidity and develop considerable skill with the technique has been described in earlier literature; Preston 1947). Despite this limitation, this study clearly indicates that New Zealand plant fibre cells exhibit noticeably lower n_{\parallel} values than their European counterparts. Therefore birefringence measures may be incorporated in the suite of techniques for determining if an artefact from New Zealand is comprised of plant material of indigenous or European origin.

Whilst the present study examined the characteristics of fibre cells of plant species considered to be the major textile species used in New Zealand, it remains possible that other plant species may have been used historically, albeit less frequently. Previous studies by Goulding (1971), Pendergrast (1996) and McCallum and Carr (2012) have suggested lesser-known plants were used in the production of woven objects (e.g. pingao *Ficinia spiralis* (Cyperaceae), houi/houhere *Hoheria* species (Malvaceae), tikumu *Celmisia* species (Asteraceae, generally *C. semicordata*; Lord *et al.* 2010)), with the latter two genera representing more than 10 separate species or subspecies. Therefore future research efforts should be focused towards not only characterising all known Māori textile plants species, but also other taxonomically related species used within New Zealand and around the Pacific. For example, there are two additional *Cordyline* species with restricted distributions in New Zealand; tī koraha/tī rauriki *Cordyline pumilio* dwarf cabbage tree (northern North Island; north of 38°S) and *Cordyline obtecta* (synonym *C. kaspar*) Norfolk Island/Three Kings cabbage tree (North Cape and offshore islands [e.g. Three Kings Islands, Poor Knights Islands], North Island, New Zealand, and Norfolk Island; Harris *et al.* 2001). Although neither species has been previously mentioned in literature pertaining to Māori textiles, it is

possible that “tī kōuka” textiles from these regions may be composed from these locally abundant *Cordyline* species. Furthermore the genus *Cordyline* is represented by up to 10 additional species throughout the western Pacific which may also be represented in the collections of cultural institutions.

As current knowledge of species used in Māori woven objects largely originates from inter-generational knowledge transferred between weavers, it remains possible that knowledge has been lost in the use of plant species with restricted geographic distributions or with less favoured physical properties. Herein lies the importance of verifying the plant species from Māori artefacts present in institutional collections worldwide, since such artefacts may hold the key to unlocking the hidden diversity of plant species used in the production of textiles, which are no longer in use by contemporary weavers. The need now is to determine whether the techniques outlined in this study provide superior characterisation of plant species composition than other analytical techniques (e.g. μ CT, Smith *et al.* 2013; SEM, Cartwright 2013) and to increase confidence in species identification through wider sampling of phenotypic variation within each species. Future research also needs to determine whether customary dye treatments, commonplace in objects held within museum collections, alter the morphological and/or birefringent characteristics of ultimate fibre cells used to identify woven Māori objects.

This technique provides two important benefits in the context of plant materials identification in museum artefacts. Very small specimens of fibre aggregate or whole leaf (2-3mm long pieces) yield abundant quantities of ultimate fibre cells from which multiple species identification from single genera may be achieved. In a context where destructive and purposive sampling directly from artefacts is problematic, this sample size, coupled with the

high confidence of accurate identification, is very appealing. The typical size of specimen required will neither alter the structural integrity nor aesthetic properties of most museum artefacts (for example raw internal woven edges of kete, or a frayed / abraded fibre of a kakahu could easily provide sufficient material for positive identification of the plant species in almost 100 percent of cases). While the use of already detached material for identification purposes is an appealing prospect in most museum contexts, the accuracy of the method described in this study, in conjunction with the small sample size required, show a way forward where direct sampling may become more acceptable to stakeholders.

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REFERENCES

Becke, F. 1893. Ueber die bestimmbarkeit der gesteinsgemengtheile, besonders der plagioklase auf grund ihres lichtbrechungs vermögens. *Sitzungsberichte der kaiserlichen Akademie der Wissenschaften, mathematisch-naturwissenschaftliche Classe*, **102**, 358-378.

Bergfjord, C. & Holst, B. 2010. A procedure for identifying textile bast fibres using microscopy: Flax, nettle/ramie, hemp and jute. *Ultramicroscopy*, **110**, 1192-1197.

Bergfjord, C., Karg, S., Rast-Eicher, A., Nosch, M. L., Mannering, U., Allaby, R. G., Murphy, B. M. & Holst, B. 2010. Comment on "30,000-Year-Old Wild Flax Fibers". *Science*, **328**, 2.

Bergfjord, C., Mannering, U., Frei, K. M., Gleba, M., Scharff, A. B., Skals, I., Heinemeier, J., Nosch, M. L. & Holst, B. 2012. Nettle as a distinct Bronze Age textile plant. *Scientific Reports*, **2**, 664.

Carr, D., Cruthers, N., Smith, C. & Myers, T. 2008. Identification of selected vegetable textile fibres. *Reviews in Conservation*, **53**(2), 75-87.

Carr, D. J. & Cruthers, N. M. 2005-2007. *Microscopy of selected plant fibres of cultural significance to New Zealand and the Pacific*, Dunedin (New Zealand), Department of Clothing and Textile Sciences, University of Otago.

Carr, D. J. & Cruthers, N. M. 2007. He rārangi whakaaturanga o nga taonga rākau. Atlas of plant material and fibres from New Zealand and the Pacific, Dunedin (New Zealand), Department of Clothing and Textile Sciences, University of Otago.

Cartwright, C. 2013. Scanning electron microscope identification of fibres and hairs from the Cook Voyage Collections at the Pitt Rivers Museum. London: British Museum.

Catling, D. & Grayson, J. 1982. *Identification of long vegetable fibres*, London, Chapman and Hall.

Eder, M. & Burgert, I. 2010. Chapter 2.2 Natural Fibres – Function in Nature. In: Müssig, J. ed. *Industrial Applications of Natural Fibres - Structure, Properties and Technical Applications*, Chichester, John Wiley & Sons. pp 23-40.

Goodman, M. A. 2009. Microscopic fiber identification by refractive index. [Available: <http://www.slideshare.net/tovman/ri-fiber-id-rev02>]

Gibson, L. J. 2012. The hierarchical structure and mechanics of plant materials. *Journal of The Royal Society Interface*, **9**, 2749-2766.

Goulding, J. H. 1971. Identification of archaeological and ethnological specimens of fibre-plant material used by the Maori. *Records of the Auckland Institute and Museum*, **8**, 57-101.

Harris, W., Beever, R. E. & Smallfield, B. 2001. Variation in response to cold damage by populations of *Cordyline australis* and of some other species of *Cordyline* (Lomandraceae). *New Zealand Journal of Botany*, **39**, 147-159.

Harris, W., S.M. Scheele, C.E. Brown, and J.R. Sedcole. 2005. Ethnobotanical study of growth of *Phormium* varieties used for traditional Maori weaving. *New Zealand Journal of Botany*, **43**, 83-118.

Haugan, E. & Holst, B. 2013. Determining the fibrillar orientation of bast fibres with polarized light microscopy: the modified Herzog test (red plate test) explained. *Journal of Microscopy*, **252**, 159-168.

Haugan, E. & Holst, B. 2014. Flax look-alikes: pitfalls of ancient plant fibre identification. *Archaeometry*, **56**, 951-960.

Herzog, A. 1955. *Mikrophotographischer Atlas der technisch wichtigen Pflanzenfasern* (2. Aufl.), Berlin, Akademie-Verlag.

Hofreiter, M., Serre, D., Poinar, H. N., Kuch, M. & Pääbo, S. 2001. Ancient DNA. *Nature Reviews Genetics*, **2**, 353-359.

Ilvessalo-Pfäffli, M.-S. 1995. *Fiber atlas: identification of papermaking fibers*, Springer Science & Business Media.

Jakes, K. A., Sibley, L. R. & Yerkes, R. 1994. A comparative collection for the study of fibres used in prehistoric textiles from Eastern North America. *Journal of Archaeological Science*, **21**, 641-650.

Liese, W. 1987. Research on bamboo. *Wood Science and Technology*, **21**, 189-209.

Long, R. L., Bange, M. P., Gordon, S. G. & Constable, G. A. 2010. Measuring the maturity of developing cotton fibers using an automated polarized light microscopy technique. *Textile Research Journal*, **80**, 463-471.

Lord, J., McCallum, R., Smith, C. A. & Carr, D. 2010. Use and identification of tikumu (*Celmisia* species, Asteraceae) in artefacts of New Zealand origin. *Journal of the American Institute for Conservation*, **49**, 69-82.

Luniak, B. 1953. *The identification of textile fibres*, London, Pitman and Sons.

Mccallum, R. E. & Carr, D. J. 2012. Identification and use of plant material for the manufacture of New Zealand indigenous woven objects. *Ethnobotany Research & Applications*, **10**, 185-198.

McCrone, W. C. 1999. 5 - Light microscopical analytical techniques. In: Lefurgey, P. I. D. S. L. R. (ed.) *Biomedical Applications of Microprobe Analysis*. San Diego: Academic Press.

McCrone, W. C. & Dell, J. G. 1973. *The particle atlas. Volume Four The particle analysts handbook*, Ann Arbor, Michigan, USA, Anne Arbor Science Publishers.

Palenik, S. J. 1999. Microscopical examination of fibres. *In*: Robertson, J. & Grieve, M. (eds.) *Forensic Examination of Fibres, Second Edition*. CRC Press.

Pendergrast, M. 1996. The fibre arts. *In*: Starzecka, D. C. (ed.) *Māori Art and Culture*. Auckland (New Zealand): David Bateman Ltd.

Petraco, N. & Kubic, T. 2004. *Color atlas and manual of microscopy for criminalists, chemists, and conservators*. Boca Raton: CRC Press.

Preston, J. M. 1933. Relations between the refractive indices and the behaviour of cellulose fibres. *Transactions of the Faraday Society*, **29**, 65-71.

Preston, J. M. 1947. 12—The measurement of the refractive indices of fibres. *Journal of the Textile Institute Transactions*, **38**, T78-T83.

Preston, R. D. 1974. *The physical biology of plant cell walls*, London, Chapman and Hall.

Prychid, C. J. & Rudall, P. J. 1999. Calcium oxalate crystals in monocotyledons: a review of their structure and systematics. *Annals of botany*, **84**, 725-739.

Schaffer, E. 1981. Fiber identification in ethnological textile artifacts. *Studies in Conservation*, **26**, 119-129.

Scheele, S. 2005. *Harakeke The Rene Orchiston Collection*. 3 ed. Lincoln, Manaaki Whenua Press.

Skoglund, G., Nockert, M. & Holst, B. 2013. Viking and early Middle Ages Northern Scandinavian textiles proven to be made with hemp. *Scientific Reports*, **3**, 2686.

Smith, C., Lowe, B., Blair, K., Carr, D. & McNaughton, A. 2013. Identification of historical plant material using micro-computed tomography. *Studies in Conservation*, **58**, 256-268.

Smith, C. A., Lowe, B. J. & McNaughton, A. 2014. Micro-computed tomography for plant identification in artefacts. *In*: Bridgland, J., ed. ICOM-CC 17th Triennial Meeting Preprints, 15-19 September 2014 2014 Melbourne, Australia. Paris: International Council of Museums, 7 pp.

Tamarapa, A. & Wallace, P. 2013. '*Māori clothing and adornment – kākahu Māori - Ngā taonga tuku iho – traditional Māori dress*', *Te Ara - the Encyclopedia of New Zealand*.

Available: <http://www.TeAra.govt.nz/en/maori-clothing-and-adornment-kakahu-maori/page-1> [2016].

Te Kanawa, D., Hanly, G., Brown, C., Te Kanawa, A., Aotearoa Moananui a Kiwa Weavers, Puwaha ki te Ao Trust. 2006. *Weaving a kakahu*, Hamilton, New Zealand, Puwaha ki te Ao Trust.

The Textile Institute. 1975. *Identification of textile materials*, Manchester, The Textile Institute.

Tomlinson, P. B. 2006. The uniqueness of palms. *Botanical Journal of the Linnean Society* **151**, 5-14.

Tridico, S., Houck, M., Kirkbride, K. P., Smith, M. & Yates, B. 2014. Morphological identification of animal hairs: myths and misconceptions, possibilities and pitfalls. *Forensic Science International*, **238**, 101-107.

Tridico, S. R. 2009. Natural animal textile fibres: structure, characteristics and identification. *In: Houck, M. M. (ed.) Identification of textile fibers*. Cambridge: Woodhead Publishing Ltd.

Valaskovic, G.A. 1991. Polarized light in multiple birefringent domains: a study of the Herzog effect. *The Microscope*, **39**, 269-286.

Nadirah, W.W., Jawaid, M., Al Masri, A.A., Khalil, H.A., Suhaily, S.S. and Mohamed, A.R., 2012. Cell wall morphology, chemical and thermal analysis of cultivated pineapple leaf fibres for industrial applications. *Journal of Polymers and the Environment*, **20**, 404-411.

Wheeler, B. & Wilson, L. J. 2008. *Practical forensic microscopy: A laboratory manual*, Hoboken, Wiley.

TABLES

Table 1. Provenance/phenotype and sampling details of New Zealand plant species assessed using polarised light microscopy.

Species	Latin name	Provenance/phenotype	Sample location	Sample year
Harakeke	<i>Phormium tenax</i>	a) Makaweroa cultivar; b) Phenotype 1 (P7); c) Phenotype 2 (P6); d) Phenotype 3 (P1)	a) Waitati, Otago, South Island; b-d) Dunedin Botanic Garden, Dunedin, Otago, South Island	a) 2014; b-d) 2007
Wharariki	<i>Phormium cookianum</i> subsp. <i>cookianum</i>	Limestone Stream, Upper Awatere Valley, Westland, South Island ¹	National New Zealand Flax Collection, Lincoln, Canterbury, South Island	2006
Wharariki	<i>Phormium cookianum</i> subsp. <i>hookeri</i>	Long Beach, Otago, South Island	Waipuna Bay, Otago, South Island	2014
Tī kōuka	<i>Cordyline australis</i>	Lower Arahura, Westland, South Island	Tī kōuka Collection, Invermay, Otago, South Island	2014
Tī ngahere	<i>Cordyline banksii</i>	Mt Te Aroha, Waikato, North Island	Tī kōuka Collection, Invermay, Otago, South Island	2014
Tī tōi	<i>Cordyline indivisa</i>	Hauhungaroa Range, Manawatu- Wanganui, North Island	Tī kōuka Collection, Invermay, Otago, South Island	2014
Kiekie	<i>Freycinetia banksii</i>	a) unknown; b) Cape Foulwind, Westland, South Island; c) Broughton Bay, Marlborough, South Island ²	a-b) Dunedin Botanic Garden, Dunedin, Otago, South Island; c) Broughton Bay, Marlborough, South Island	a-b) 2014; c) 2006

Notes: ¹Specimen 2WhiWhB and ²4_KieKie_July_06 from Carr and Cruthers 2005-2008

FIGURES

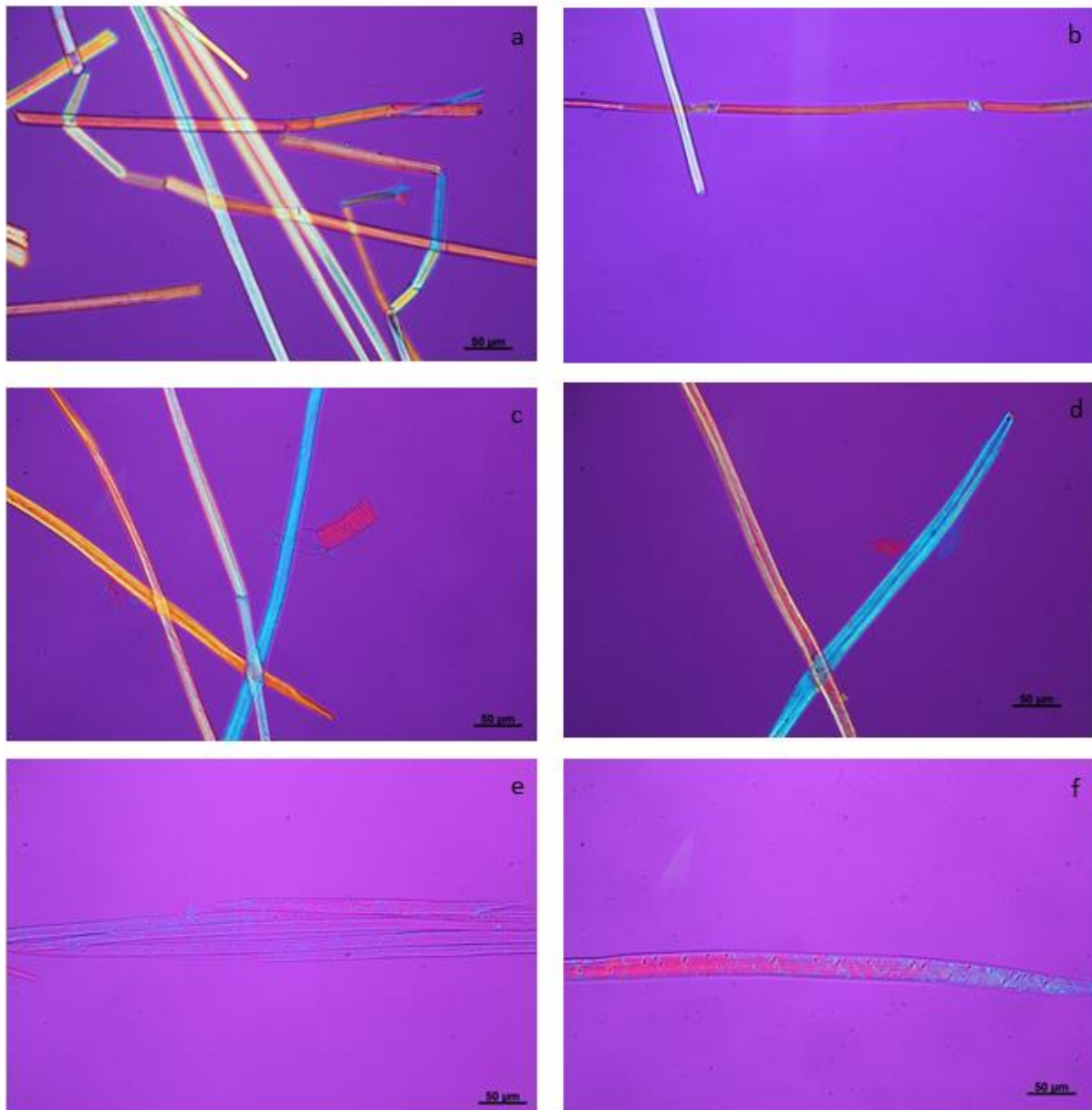


Figure 1. Variation in fibre cell wall pits abundance among New Zealand plant species utilised in Māori textiles; low abundance - (a) harakeke Makaweroa *Phormium tenax*, (b) wharariki *P. cookianum* subspecies *cookianum*; moderate abundance – (c) tī ngahere *Cordyline banksii*, (d) tī kōuka *C. australis*; high abundance – (e) kiekie *Freycinetia banksii*, (f) tī tōi *C. indivisa*.

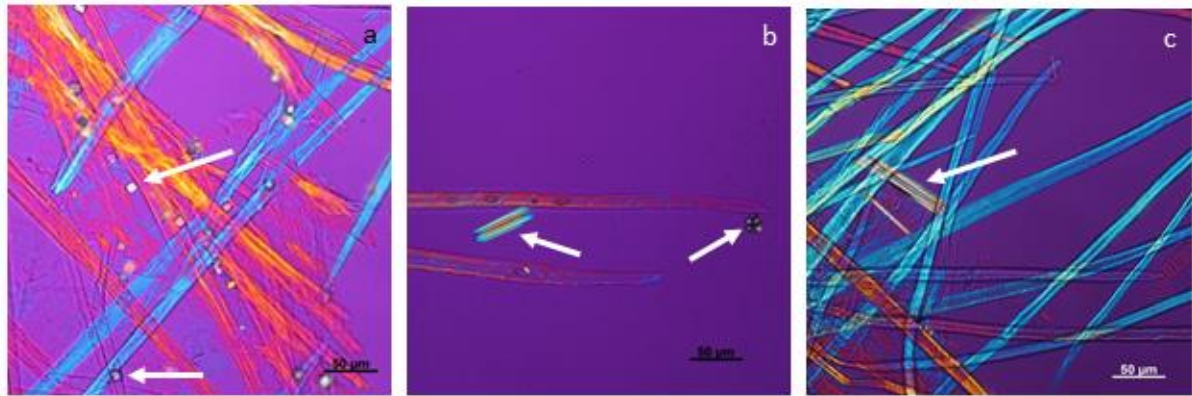


Figure 2. Variation in crystal shapes associated with New Zealand plant species utilised in Māori textiles; (a) cuboidal styloids - kiekie *Freycinetia banksii*; (b) elongated styloids and conglomerate – tī kōuka *Cordyline australis*; (c) raphide – tī ngahere *C. banksii*. Arrows indicate crystal position.

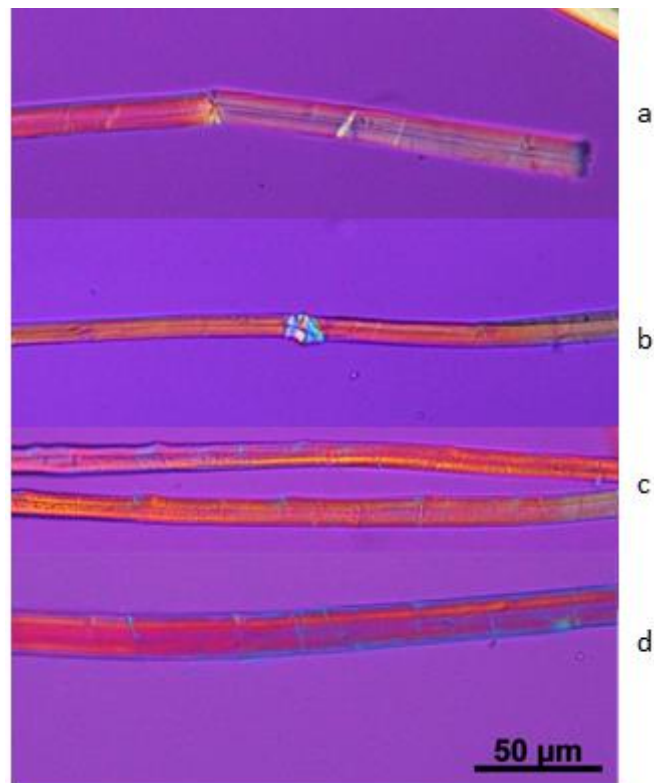


Figure 3. Cross-markings and dislocation of fibre cells among New Zealand plant species utilised in Māori textiles; (a) harakeke Makaweroa *Phormium tenax*, (b) wharariki *P. cookianum* subspecies *cookianum*, (c) wharariki *P. cookianum* subspecies *hookeri*, (d) tī ngahere *Cordyline banksii*.

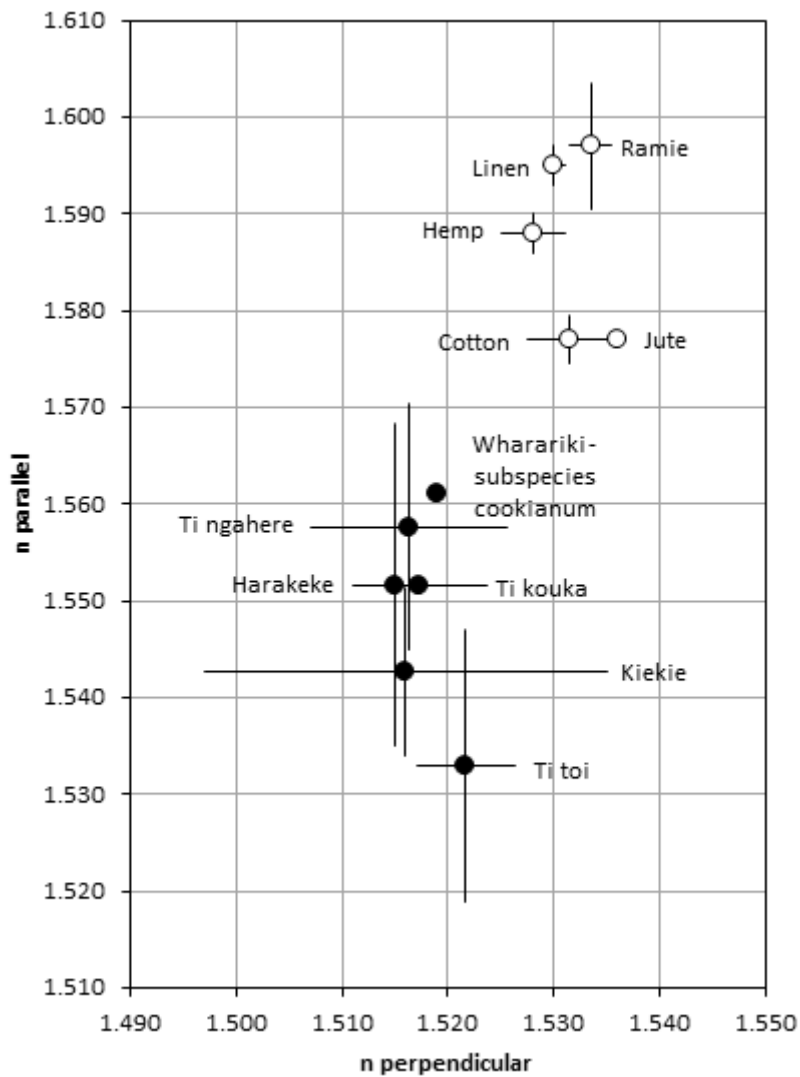


Figure 4. Comparison of parallel and perpendicular refractive indices (n_{\parallel} and n_{\perp}) of fibre cells from New Zealand (dark circles, this study) and European plant species (open circle; Luniak 1953). Error bars represent variance.

SUPPLEMENTARY FILES

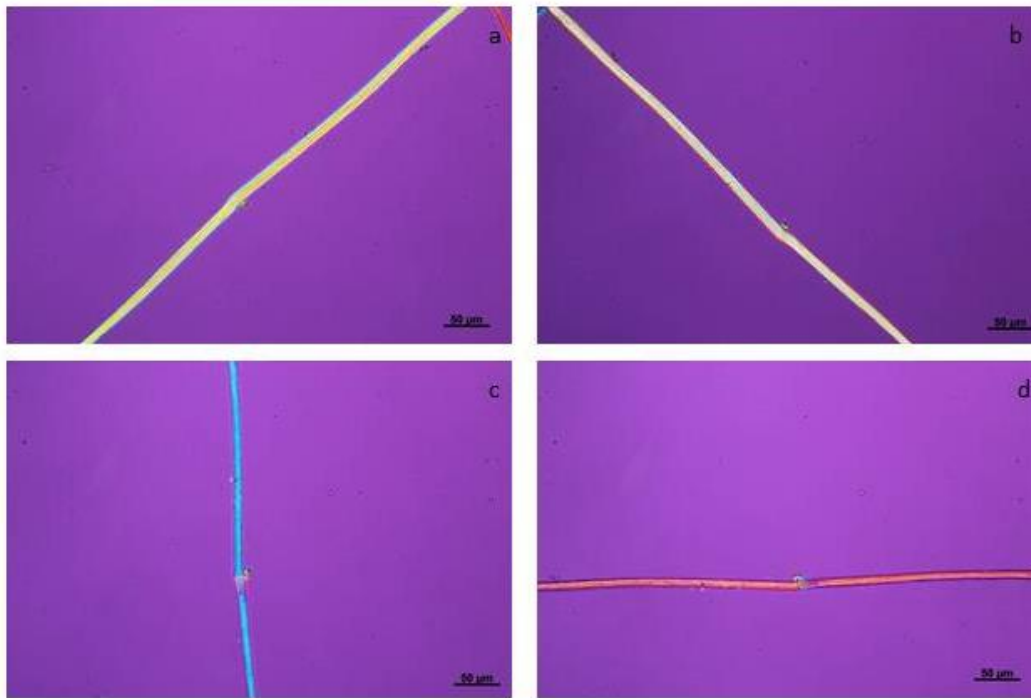


Figure S1. Harakeke - *Phormium tenax*, Makaweroa cultivar; a) sign of elongation SE $\alpha = 45^\circ$, b) SE $\alpha = 135^\circ$, c) Herzog $\alpha = 0^\circ$, d) Herzog $\alpha = 90^\circ$.

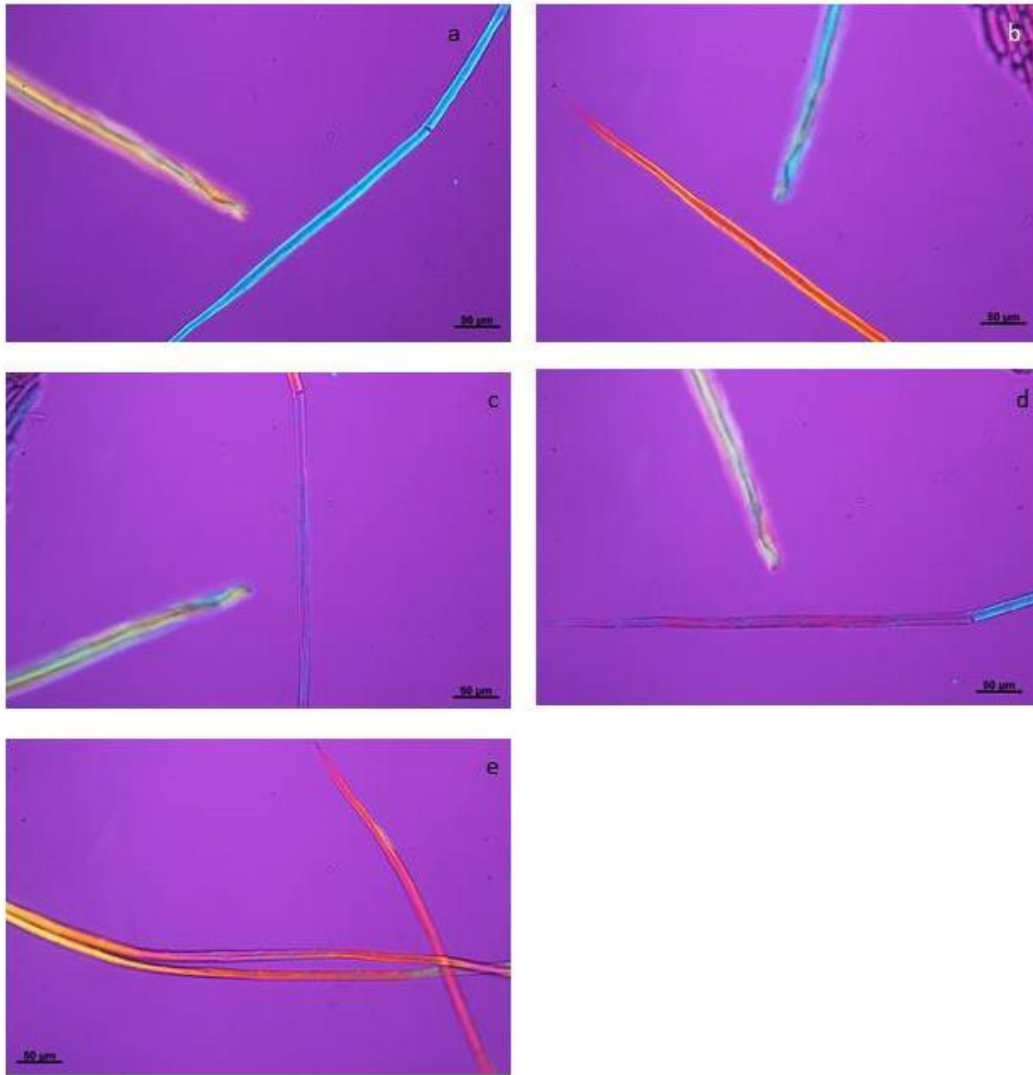


Figure S2. Wharariki - *Phormium cookianum* subsp. *hookeri*; a) sign of elongation SE $\alpha = 45^\circ$, b) SE $\alpha = 135^\circ$, c) Herzog $\alpha = 0^\circ$, d) Herzog $\alpha = 90^\circ$, e) whole cells.

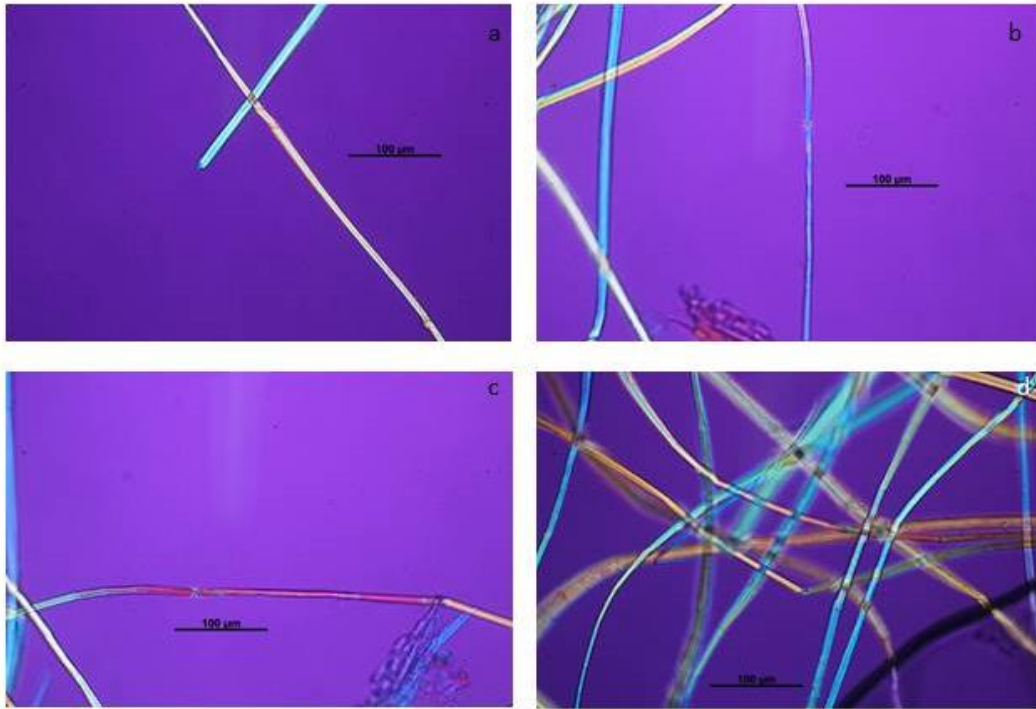


Figure S3. Wharariki - *Phormium cookianum* subsp. *cookianum*; a) sign of elongation SE $\alpha = 45^\circ$ and 135° , b) Herzog $\alpha = 0^\circ$, c) Herzog $\alpha = 90^\circ$, d) whole cells.

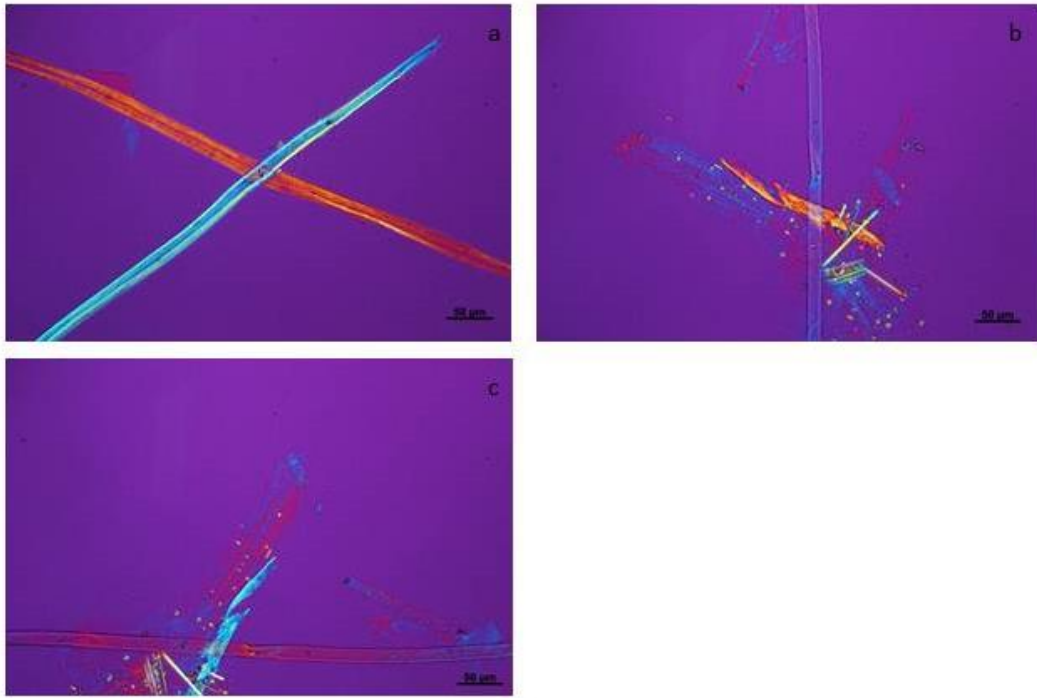


Figure S4. Tī kōuka – *Cordyline australis*; a) sign of elongation SE $\alpha = 45^\circ$ and $= 135^\circ$, b) Herzog $\alpha = 0^\circ$, c) Herzog $\alpha = 90^\circ$.

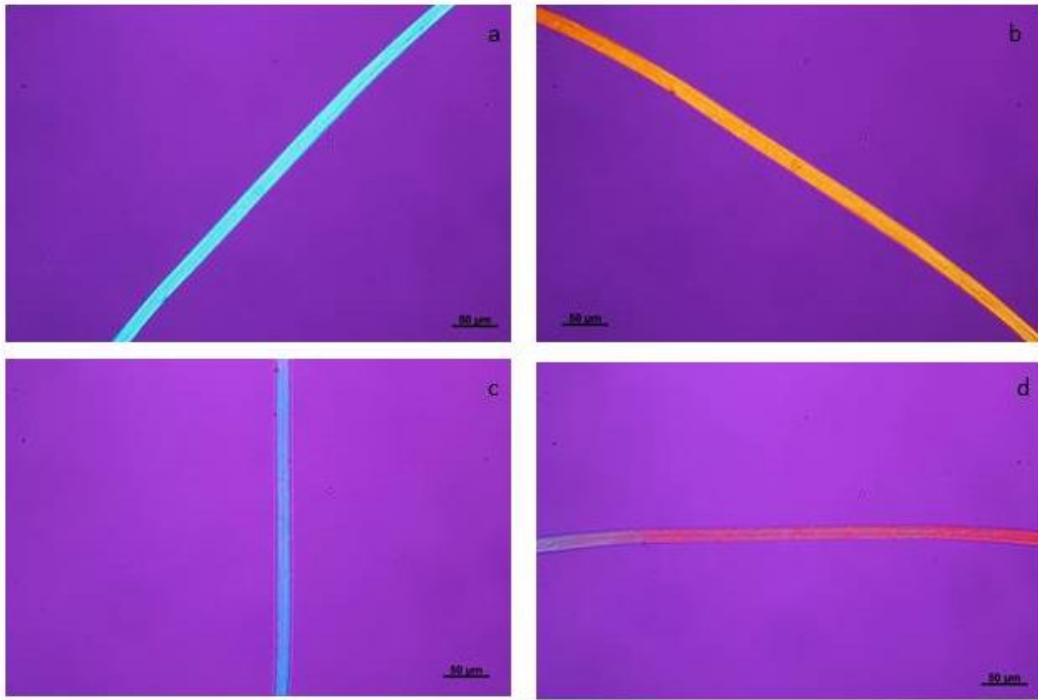


Figure S5. Tī ngahere – *Cordyline banksii*; a) sign of elongation SE $\alpha = 45^\circ$, b) SE $\alpha = 135^\circ$, c) Herzog $\alpha = 0^\circ$, d) Herzog $\alpha = 90^\circ$.

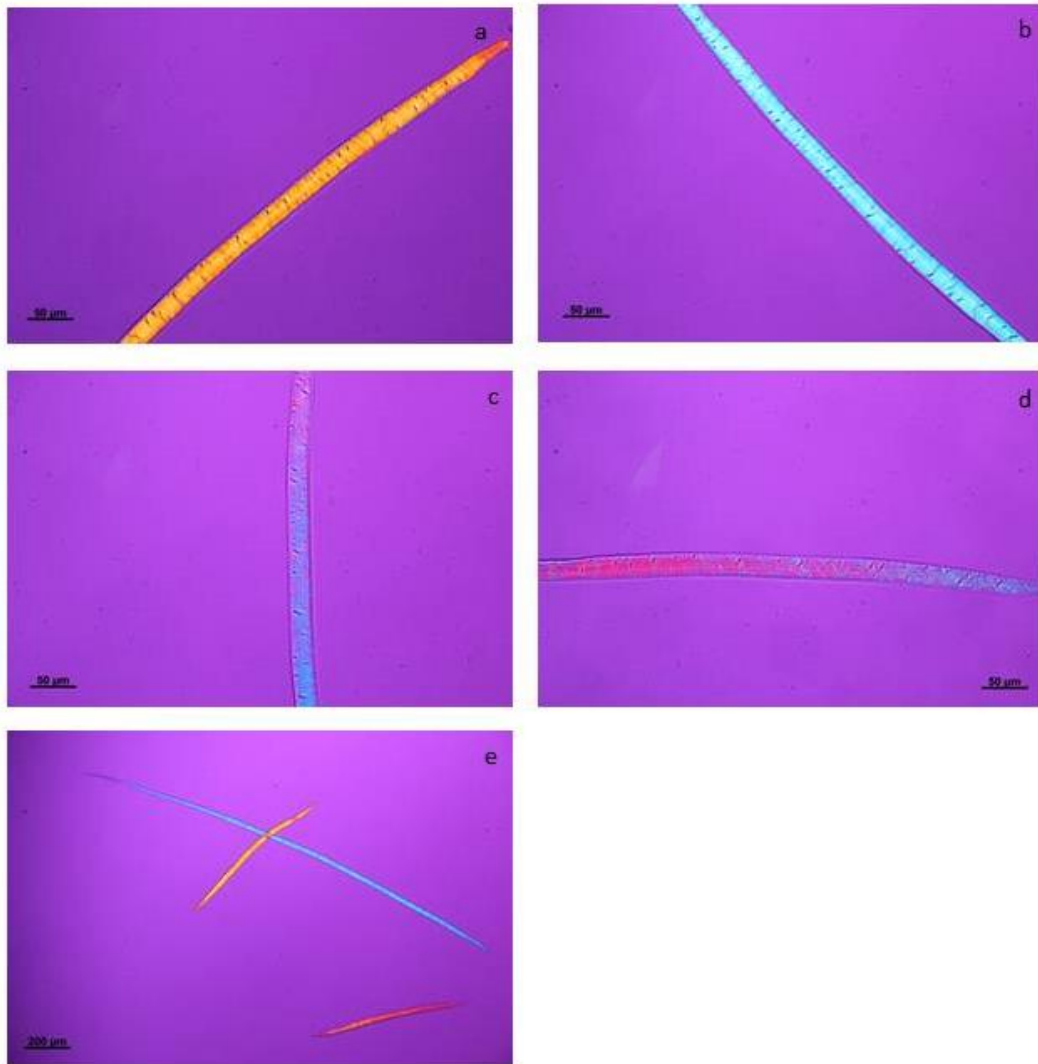


Figure S6. Tī toi – *Cordyline indivisa*; a) sign of elongation SE $\alpha = 45^\circ$, b) SE $\alpha = 135^\circ$, c) Herzog $\alpha = 0^\circ$, d) Herzog $\alpha = 90^\circ$, e) whole cells.

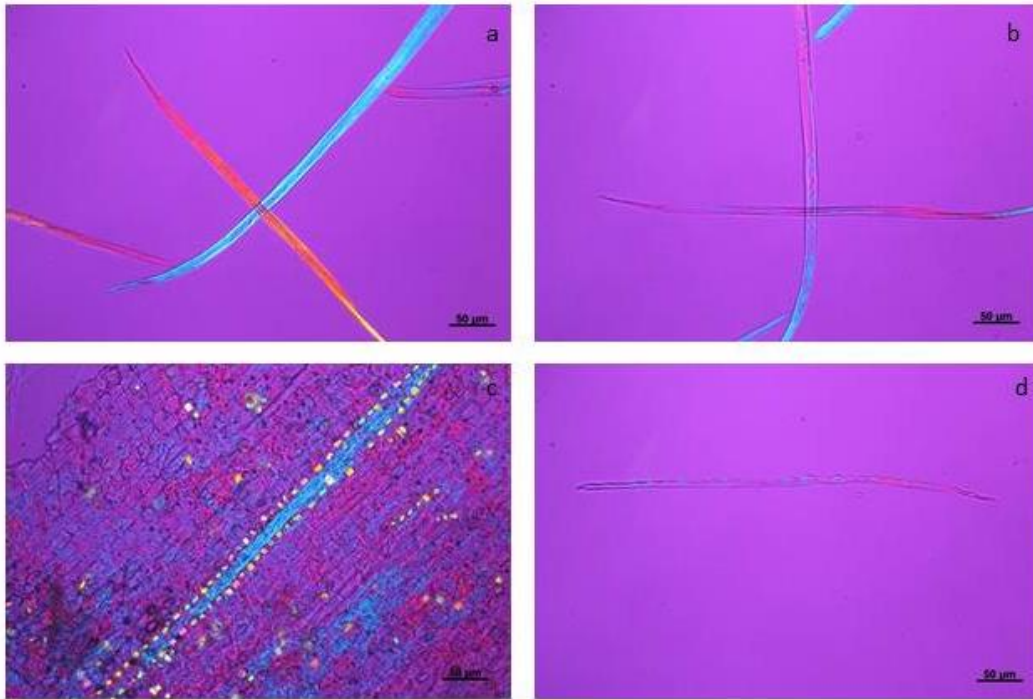


Figure S7. Kiekie – *Freycinetia banksii*; a) sign of elongation SE $\alpha = 45^\circ$ and 135° , b) Herzog $\alpha = 0^\circ$ and 90° , c) cuboidal styloid crystal alignment, d) scalloping.