IDENTIFICATION OF HISTORICAL PLANT MATERIAL USING MICRO-COMPUTED TOMOGRAPHY

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IDENTIFICATION OF PLANT MATERIAL FROM ARTEFACTS USING MICRO-COMPUTED TOMOGRAPHY

ABSTRACT [HEADING]

This work investigates the use of micro-computed tomography (micro-CT, μCT) for identification of New Zealand plant leaf material from artefacts. Micro-computed tomography was explored as a result of difficulties in preparing transverse sections from aged plant material from artefacts to compare with reference slides for microscopic identification of plant species. The three plants investigated (harakeke[1], New Zealand flax, *Phormium tenax*, J.R.Forst. & G.Forst; tī kōuka, cabbage tree, *Cordyline australis*, (Forst.f.) Endl,; kiekie, *Frecinetia banksii* A.Cunn.) were/are commonly used by Māori for the manufacture of objects often found in cultural institutions. Contemporary and historical specimens (from artefacts) of plant leaf material were investigated. Contemporary specimens were viewed using micro-CT and showed identifiable features compared with micrographs of transverse sections from reference material. Diagnostic features of each plant species were then named and measured, providing the basis for development of an identification key using both visual and objective criteria. Positive identification of historical specimens using this key varied across plant species and according to level of ageing and processing. Despite this, micro-CT had several advantages over traditional transverse sections: samples were not prepared for, or altered by, analysis, and numerous cross-sections across the entire sample could be easily viewed to locate identifiable characteristics. While measurable criteria supplied apply only to the three named New Zealand plant species, this paper provides methods that could be applied to the identification of

[1] Plant names in te reo Māori (Māori language) are followed by the English name and botanic name, and are subsequently referred to by Māori names.

other aged plant leaf material. Knowledge of plant anatomy at the level of major cell and tissue types (for e.g. parenchyma, sclerenchyma and epidermis) is sufficient for the level of analysis carried out in this study.

INTRODUCTION [HEADING]

Plants used by Māori to manufacture clothing, baskets, nets, snares, and mats include harakeke (New Zealand flax, *Phormium tenax* J.R.Forst. & G.Forst), tī kōuka (cabbage tree, *Cordyline australis* (Forst.f.) Endl) and kiekie (*Freycinetia banksii* A.Cunn.) [1-5]. The positive identification of such plant materials used to manufacture artefacts held by cultural institutions can be difficult. Artefacts may be dirty, or have undergone conservation treatments, masking distinctive morphological features that have been commonly examined using microscopy to identify the source of the plant material [6, 7]. Alternatively, morphological features may have been eroded due to processing, handling, or degradation.

The removal of material from the artefact, or the use of fragments previously detached from the artefact, for the manufacture of transverse-sections may not be deemed acceptable for ethical or cultural reasons. Even when it is acceptable to take a sample of material from an artefact, the preparation of transverse-sections from aged (embrittled, processed) plant material is very difficult, even for an experienced practitioner. For diagnostic certainty it is desirable that multiple transverse sections be taken, and slides prepared from them, along the entire length of an unknown plant sample. However the size and embrittlement of specimens of plant material sampled from artefacts makes this problematic. Additionally a transverse section may be taken from an area where diagnostic characteristics have been destroyed by age or processing: this only becomes evident after the investment of time and resources in sectioning and slide preparation.

Micro-computed tomography (micro-CT) can be used to examine small fragments of plant material with little or no preparation of the sample, enables multiple internal regions across an entire sample to be viewed and is non-destructive [8-14], and is therefore of interest to conservators.

This paper examines the use of micro-CT to

a) distinguish morphological features for identification of selected plant species (harakeke, tī kōuka, kiekie) historically used by Māori, and

b) identify plant leaf material from artefacts (aged, processed) made by Māori.

IDENTIFICATION OF PLANTS USED BY MĀORI [HEADING]

Plant materials commonly used by Māori for the production of dress and other textiles include harakeke, tī kōuka and kiekie [5], all of which are endemic to New Zealand, with harakeke also endemic to Norfolk Island [5, 7]. Most plants (including these three species) used for Māori textile production are monocotyledons [15]. Monocotyledons are a type of flowering plant that have only one seed leaf, and mature leaves with long, parallel vascular and fibre bundles [7, 16](Figure 1). This means the defining features of many plants used to make Māori textiles, such as vascular bundle size and shape, and the pattern they form across the leaf, can often be very similar, making it difficult to distinguish among species.

The identification of plant materials commonly relies on comparison of transverse sections with a set of reference images of a known sample of plants, clearly showing identified diagnostic characteristics [17, 18]. Carr and Cruthers [7] present a set of reference transverse sections of New Zealand and Pacific plant species including harakeke, tī kōuka and kiekie, showing distinctive features, such as

vascular bundle size and shape, as well as pattern repetition. Other distinguishing characteristics are ultimate fibre measurements (diameter and length), the existence of crystals within leaf tissues, and surface characteristics of these plants, including the existence and morphology of wax[19]. The first aim of this project was to determine whether images gained from micro-CT displayed the morphological features used for identification of New Zealand plant materials visible in more traditional transverse sections.

While reference material can be invaluable in the identification of plant materials, materials in artefacts are likely to be altered by processing, use and age [18, 20]. Archaeological material can be brittle, dry and covered with soil, which may affect the ability to make cross-sections and examine surface characteristics [21]. The second aim of the project was therefore to determine whether micro-CT was better able to distinguish morphological features for identification of aged, processed plant material from artefacts made by Māori, than viewing micrographs of transverse sections. The minimal preparation of samples required for micro-CT (sectioning using a microtome for the preparation of transverse sections itself potentially causes distortion and damage to remaining morphological features in samples from artefacts) and whilst enabling multiple cross sections for each sample to be viewed, even when very small and brittle ,were thought likely to be advantageous.

COMPUTED MICROTOMOGRAPHY [HEADING]

Tomography uses X-rays to image an object in sections [22]. The technique is most commonly recognised in medicine as computed axial tomography (CAT) or computed tomography (CT) scanning. Section data are collected by an X-ray source and detector that move around the object, and are reconstructed after scanning to create a three-dimensional model of the object, which itself can be

sectioned to view internal structures. The technique is non-invasive and non-destructive (e.g. [23]Sasov and van Dyck 1998). Micro-CT scanning produces models with detection (1 μm) and resolution (2-3 μm), and is used on small specimens (maximum diameter range typically 70mm). As micro-CT is a non-invasive technique that can generate an image of the internal structure of an object [9, 23, 24], it has a wide range of applications, including the examination of plant anatomy, bone, food and tissue cultures [8, 10-14, 25-27].

The conservation literature reports the use of CT scanning to identify complex multi-media artefacts, particularly from archaeological contexts [28-31], and notes the potential of micro-CT for examination of finer aspects of textiles, such as individual yarns and fibres [29]. As studies have shown that micro-CT images can have similar spatial resolution and detail to optical micrographs [8, 11], and given the difficulties inherent in preparing transverse sections for micrographs already outlined, it was decided to test micro-CT as a means for identifying plant species of importance in New Zealand.

PRINCIPLES OF MICRO-CT [HEADING]

Micro-CT involves the projection of X-rays at an object, the detection of X-ray transmission through the object, and reconstruction of the data into two (2D) and three-dimensional (3D) images of the object [9, 23, 24, 31]. X-rays are a form of high-energy electromagnetic radiation (light). X-ray transmission is characterised by the Beer-Lambert Law:

$$I = I_o e^{-\mu x}$$

Where I is the transmitted X-ray beam intensity, I_o is the initial beam intensity, x is the thickness of material the X-ray beam traverses, and μ is the linear attenuation coefficient [30]. The intensity of transmitted X-rays is thus dependent on the

energy of generated X-rays, sample thickness and the linear attenuation coefficient of the respective materials within a sample. The linear attenuation coefficient is a measure of the fraction of X-rays that are absorbed or scattered per unit thickness of a material, and differs depending on the density of the material [30].

Scanning an object with X-ray beams over a series of angles (achieved by rotating the object between a static source and detector), generates accurate representations of the internal structure of the object. Differences in material density correspond to differences in attenuation of X-rays recorded by the detector. The data received by the detector are processed using relevant computer algorithms into 2D cross-section images, which can then be stacked using relevant software to create 3D reconstructions of the object [8, 9, 11-14, 25-27].

METHODS [HEADING]

DIAGNOSTIC FEATURES [SUB-HEADING]

In order to distinguish morphological features for identification of selected plant material (harakeke, tī kōuka, kiekie) historically used by Māori, a suite of key diagnostic features and measureable characteristics was developed (Table 1) by examining previously published transverse sections of each species [7](Figure 2). Where possible, cross-sections of more than one individual plant were examined, in order to assess the likely variability of features within a species (Table 2). The shape and appearance of vascular bundles, fibre bundles (which are components of vascular bundles), mesophyll and epidermal tissues were noted for each species (Figure 2). In general, harakeke and tī kōuka vascular bundles consisted of a central round or oval region of vascular tissue capped by fibre bundles. These vascular and fibre bundles were arranged in repeating patterns along the top (abaxial) and bottom (adaxial) of the leaf (Figure 2(a) and (e)). Fibre bundles in

harakeke appeared to be long, thin and tapered, while tī kōuka appeared shorter and wider, with a squarer shape. Vascular bundles in kiekie consisted of an ellipse-shaped conglomerate of sclerenchyma tissue (fibre), with a round or oval region of vascular tissue in the centre (Figure 2 (c)).

The shapes of fibre bundles were classified as molar tooth (MT), keyhole (KH) [7], intermediate (intermediate between molar and keyhole; IM), ellipse (kiekie only) or satellite (not associated with a central vascular bundle) for the process of identification (Figure 2).

SAMPLES [SUB-HEADING]

Two types of plant material specimens were then used for the investigation of micro-CT as an analytical tool for plant materials identification. Samples of dried, contemporary leaf material were provided by Manaaki Whenua, Lincoln, New Zealand, or leaves were collected by the authors from plants growing in the Dunedin Botanic Garden. Natural variability expected within each species was investigated as follows. Two harakeke plants, known from previous work[32] to represent contrasting extremes of fibre bundle size and shape, were selected and a leaf from each sampled. The variability within kiekie and tī kōuka is not as well understood. Therefore three plants each of kiekie and tī kōuka, originating from widely separated geographic regions were selected and a leaf of similar age sampled from each (Table 2). For leaf samples specimens (approximately 5mm x 10mm) were cut from the centre portion of the blade of each leaf using a scalpel, and oven dried at 50 ^{0}C for 3 days at the Department of Applied Sciences, Clothing and Textile Sciences, University of Otago.

Samples of aged, processed material were from Māori textile artefacts catalogued as made from harakeke (New Zealand flax), tī kōuka (cabbage tree) and kiekie in

the collections of the Auckland War Memorial Museum Tamaki Paenga Hira, Canterbury Museum and the National Museum of New Zealand Te Papa Tongarewa (Table 3). Samples for study were not taken directly from artefacts, rather they were small pieces (ranging in length from 1-25mm, width 0.5-3mm) that had detached over time, found in storage containers. Most New Zealand museums would prefer to not take purposive samples from artefacts for cultural and spiritual reasons, as even the smallest fragments can themselves be considered cultural artefacts (for a full discussion of taonga, and implications for research see [33]). There are obviously problems with using such samples for plant species identification; there is no guarantee that the samples provided come from the intended artefact. Additionally institutional species identification was based on catalogue records, themselves based solely on subjective means of identification, and using English descriptive terminology, rather than Māori names. This is problematic because the English name 'cabbage tree' refers to two possible species, *Cordyline australis* (tī kōuka) and *Cordlyine indiviisa* (tōī).

SAMPLE PREPARATION FOR SCANNING [SUB HEADING]

The largest fragment in each aged sample was chosen for examination. Each specimen was prepared for scanning by mounting it between two supporting pieces of open-cell polyethylene foam, and inserting into a polyethylene tube (diameter c.7mm). Where possible, a small section of the specimen was left protruding beyond the mounting media, to minimise the contribution of the polyethylene to the scanned image, and possible attenuation of low energy X-rays. The polyethylene tube was slid over a 4mm metal rod specimen holder and secured with Parafilm® (Figure 3).

MICRO-CT DATA COLLECTION [SUB-HEADING]

A Skyscan 1172 micro-CT scanner housed in the Otago Centre for Confocal Microscopy (OCCM) was used for scanning of specimens. X-rays were generated with an X-ray tube at an acceleration voltage of 38-40kV and a current of 240-250μA. Specimens were scanned over 180˚ with a rotation step of 0.4˚. X-rays were detected by a 10 μpixel 12 bit CCD Camera, capable of a 0.9 μm pixel size at maximum detector magnification. Raw X-ray attenuation data was reconstructed into datasets consisting of sequential 2D cross-sections, using the Feldkamp cone-beam back-projection algorithm available in the software NRecon [34]. Ring artefact reduction and image contrast were optimised automatically using functions available in NRecon. Images were then visually checked and histograms altered manually to improve contrast if necessary using the same software. Four combinations of Skyscan 1172 settings for detector position and camera resolution were trialled on one contemporary specimen of each species to evaluate settings producing an optimum image; i.e. showing adequate resolution of features, from a sufficiently large portion of the specimen, while minimising scan times, cost of scanning and data storage. Image resolutions ranged from 1 to 5 μm per pixel. Minimum scan time achieved per specimen was approximately 36 minutes. Images were analysed (details below) to enable selection of Skyscan 1172 settings appropriate for all samples. A micro-CT image resolution of better than 5 μm per pixel was found necessary to identify diagnostic features visible in light micrographs, whilst a resolution of 1.3 μm per pixel enabled the smallest feature, the epidermis, to be resolved for harakeke and tī kōuka (thickness typically 11 ± 4 μm in harakeke, 9 ± 2 μm in tī kōuka). The epidermis of kiekie however, could not be resolved even at the highest combination of detector and camera settings. Repeated measurements of the same features of one contemporary sample,

captured at different image resolutions, indicated no improvement in precision of measurements between different camera settings (image size 1048 x 2000 vs 2096 x 4000 pixels) at the highest detector resolution. As increasing image size doubled data capture time with no gain in measurement precision, all subsequent specimens were scanned with settings giving an image size of 1048 x 2000 pixels at 1.3 μm per pixel resolution.

DATA ANALYSIS [SUB HEADING]

Once the most appropriate micro-CT image resolution was determined, each contemporary specimen was scanned, and from the reconstructed data, a single 2D image slice selected. The dimensions of diagnostic features for each species (Table 1) were measured from these images using the ImageJ software package [35]. Measures of the shapes of features were obtained by calculating length to width ratios. Descriptive statistics (mean, standard deviation, minimum and maximum) were calculated for each species by pooling vascular bundle data across specimens (n = two, three and three specimens for harakeke, tī kōuka and kiekie respectively, giving n= 18, 17 and 14 vascular bundles for harakeke, tī kōuka and kiekie respectively). Differences in the size and shapes of features among the species were then analysed by analysis of variance routines available in the software package SPSS [36]. All data were checked for normality and homogeneity of variances prior to analysis, and were natural log transformed where variances were inhomogeneous.

An identification key was developed to aid in plant species identification. Visual diagnostic characteristics identified for each species underscored the similarity of tī kōuka and harakeke, and also that visual identification was subjective, particularly for plants, displaying high natural variability. The identification key provided a

set of dichotomous (yes/no) decisions in a hierarchical process, based on both visual and measurable characteristics visible in micro-CT images (Figure 4).

Micro-CT images of processed, aged specimens from museum samples were then captured using the same image resolution as for contemporary specimens, and where possible dimensions of diagnostic features were again measured using ImageJ software. The identification key was then trialled as a method for identifying species based on the features present in each specimen.

RESULTS [HEADING]

Most diagnostic morphological features typically visible in transverse sections (light microscopy) of the three plant species (harakeke, tī kōuka, kiekie) could be identified in high resolution (1.3 μm per pixel) micro-CT images of contemporary specimens (Figure 2). Features difficult to distinguish were the epidermis of kiekie, and the smaller satellite bundles in harakeke and tī kōuka.

Several methods were used to distinguish among contemporary specimens of the three species. These included visual recognition and measurement of features, and use of an identification key. Kiekie had specific visual characteristics distinguishing it from tī kōuka and harakeke, despite variability within kiekie in size and arrangement of features (Figure 2 and Figure 5). In particular, kiekie vascular bundle arrangement appeared as a single feature rather than a pair of upper and lower fibre bundles. The mesophyll was denser and present as discrete bands at the upper and lower leaf surface. Analysis of measurements of identified diagnostic features showed that despite the variability, particularly of kiekie, some parameters differed significantly ($p < 0.05$) among species (Figure 5 and Table 4), providing a means to assist with identification. For example, although vascular bundles in both harakeke and tī kōuka consist of pairs of upper and lower fibre

bundles, the size (length and width) and shape (ratio of length to width) of the fibre bundles differed significantly between the two species (Figure 5 and Table 4). Regardless of whether fibre bundles were upper or lower, they were always shorter in tī kōuka than harakeke. The key for identification combined visual recognition and measurements of these diagnostic features to identify species through a process of elimination (Figure 4). Provided that sufficient diagnostic features were present and measurable (in particular, both upper and lower fibre bundles in harakeke and tī kōuka), the key could be used to discriminate among the three species, despite visual similarities between harakeke and tī kōuka.

Micro-CT images of specimens from artefacts showed morphological features visible in contemporary specimens were also recognisable. However, processing and aging of material altered diagnostic features in a number of ways and with varying severity. For example, only five of the 25 specimens were complete cross-sections of leaf. In the remaining specimens, leaf surfaces were not always present, and vascular and fibre bundles, if present, were often displaced, distorted, cracked, incomplete, or appeared in isolation from any other recognisable tissues. The arrangement and apparent density of mesophyll tissue could also be displaced or distorted. Specimens exhibited a variety of combinations of these effects (Figure 6). In most cases, if vascular and/or fibre bundles were present, alteration had affected the ease of recognition and measurement of these diagnostic features (for example Figure 6 (c) has all diagnostic features present but with damage, and therefore with implications for accuracy of measurements. Most of the measures indicate tī kōuka, but one measure indicates harakeke). In specimens where bundles were cracked but could still be recognised, bundle dimensions could be estimated by addition of measurements from relevant portions (for example Figure 6 (b)). Bundles that were displaced or distorted could be measured accepting that

these measurements were most likely over-estimates of bundle dimensions (for example Figure 6 (b) and (d)). In seven of the 25 specimens examined only one leaf surface and associated fibre bundles or portions of fibre bundles were present. In these cases, it was difficult to determine whether fibre bundles were from the upper or lower portion of the leaf. Measurements therefore had to be compared with both upper and lower relevant dimensions, with ratios of OW/IW (defined in Table 1) the most useful for discriminating between harakeke and tī kōuka (Figure 5).

Despite the fragmentary and damaged nature of the specimens derived from artefacts, use of the key enabled 12 of the 25 specimens to be positively identified as either harakeke, kiekie or tī kōuka (Table 5). Kiekie proved to be the easiest to identify (seven positive identifications) followed by harakeke (four positive identifications) with tī kōuka the most difficult species to identify from aged specimens (one positive identification). In some cases, the identification differed to the species expected from the provenance of the artefact (e.g. specimens TH3, AT3 and CK3 illustrated in Fig 6g), providing evidence of the difficulties of sampling from storage containers, rather than directly from artefacts, or that catalogue information was incorrect. Some of the specimens that could not be identified in any way showed unusual features not seen in any of the three species (e.g. AH3, AK3, AT1, TT3). These specimens could represent other species. For example *Cordyline indiviisa*, mountain cabbage tree, was used for particular types of artefacts, such as *kahu tōī*, a prestigious black-dyed rain cape [37]. Two specimens were derived from artefacts labelled *kahu tōī*, but as their catalogue description in English was 'cabbage tree', they were provided for analysis. Another possibility is that these specimens were derived from other parts of the plant of the investigated

species (mid-rib or margin material which is likely to differ anatomically to the leaf blade; kiekie and tōī mid-rib were reportedly used in artefacts [38].

None of the seven specimens suspected to be tī kōuka could be positively identified as tī kōuka (the one positive tī kōuka identification was of a sample labelled harakeke). One specimen was positively identified as harakeke, two could have been either harakeke or tī kōuka, one was possibly kiekie, and three either had features that did not match any of the features in leaf cross-sections of harakeke, kiekie or tī kōuka, or the specimen was so small that the image was too indistinct to resolve any features (AT2) (Table 5). These results illustrate that plant leaf material identification can be a process of methodical elimination, rather than of instant recognition of species.

DISCUSSION [HEADING]

Only the most commonly-used plant species found in Māori artefacts were included in this preliminary study, to enable micro-CT imaging to be trialled in combination with development and testing of an identification key. Sample sizes were therefore small (harakeke n= two, kiekie and tī kōuka n= three) however this was offset by selection of cultivars of harakeke known to have large differences in appearance of fibre bundles, and by selecting tī kōuka and kiekie plants that came from disparate geographic regions. Historical samples were also selected on the basis of geographic diversity in an attempt to cover the widest possible range of variability. Further studies will aim to broaden the number of samples studied to ensure the utility of identified diagnostic features.

As in all areas of plant leaf material identification, best results were gained when the examiner of images had familiarity with contemporary specimens and the

appearance of qualitative visual features in the chosen imaging method (e.g. the absence of visible epidermis in kiekie, the differences in uniformity of density of mesophyll among species). The identification key was an important aspect of successful species identification with the understanding that when any doubt about the validity of a yes/no response existed, relevant measured characteristics must be consulted.

While preparation of the sample is very straightforward for micro-CT, like all analytical techniques practitioner experience is beneficial for data collection and analysis, including the ability to identify scanning artefacts (e.g. streaking, a typical ring artefact that distorted or disguised features), and differences between mounting materials used (polyethylene foam) and some plant material features, such as the epidermis. For a good scan result it was important that the axis of the sample was not at an angle to the axis of rotation of the instrument, as this distorted the size and shape of internal features of the specimens. This particular scanning error could be remedied by re-slicing, an image processing technique that establishes a new set of axes through the 3-D data.

CONCLUSIONS [HEADING]

Micro-computed tomography could be used to distinguish morphological features for identification of selected plant species (harakeke, tī kōuka, kiekie) historically used by Māori, and also be used for identification of plant material from artefacts made by Māori. Because of similarity between two of the plant species (harakeke, tī kōuka) visual analysis of images alone was insufficient for confident identification. Therefore a suite of measureable characteristics was also developed. An identification key was designed which combined both visual and measured characteristics to accurately identify both contemporary and historical New

Zealand plant leaf material. Difficulties in identifying historical plant leaf material from Māori artefacts were probably due to a number of factors; processing and alteration of diagnostic features during artefact production; general deterioration and ageing; the natural variability of plant species and non-purposive sampling. Despite these difficulties, micro-CT provided many advantages for plant material identification. Once optimal settings have been determined micro-CT is a relatively straightforward analytical technique. Additionally micro-CT only requires the use of small samples, and can obtain multiple section views with ease, thus providing more opportunities for the discernment of identified diagnostic characteristics; an extremely desirable ability when using samples of aged, processed material. Perhaps most importantly, particularly in specific cultural environments, the use of micro-CT as a plant identification tool enables the return of the sample in a completely unaltered state.

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MATERIALS AND SUPPLIERS [HEADING]

Contherm Thermotec 2000 Oven: Contherm Scientific Ltd, 27 Cornish St, Petone, PO Box 30-605 Lower Hutt, New Zealand. Website: www.contherm.com

ParafilmM®: MicroAnalytix Pty Ltd, POBox25-9255, Greenmount, 1730, Auckland, New Zealand, www.microanalytix.co.nz

Polyethylene foam: Dunlop Flexible Foams, 83 Harris Road, East Tamaki, Auckland, New Zealand, www.dunlopfoams.co.nz

SkyScan 1172: Skyscan, Kartuizersweg 3B, 2550 Kontich, Belgium

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corresponds to an image where a section contains upper and lower epidermis with all tissues in between. For all criteria, if unsure go to measurements (Table 4).

FIGURE 5 Box plots for all characteristics of leaf cross-section that were significantly different between three plant species: H (harakeke), K (kiekie) and T (tī kōuka). All measurements (as described in Table 3) are in μm except VB that is in number of vascular bundles per mm. Each box plot shows the median (middle horizontal black line), the lower and upper quartiles or 25% of values above and below the median (box), the range of values (the black vertical line) and the outliers (the empty dots). Measurements for kiekie were *BL, ** CW, and *** CW/EW.

FIGURE 6 Typical micro-CT cross-sections, showing increasing difficulty of classification using identification key