Grana

ISSN: 0017-3134 (Print) 1651-2049 (Online) Journal homepage: http://www.tandfonline.com/loi/sgra20

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To cite this article: Ping Li & John R. Flenley (1999) Pollen texture identification using neural networks, Grana, 38:1, 59-64, DOI: 10.1080/001731300750044717

To link to this article: http://dx.doi.org/10.1080/001731300750044717

Grana 38: 59–64, 1999

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Li, P. & Flenley, J. R. 1999. Pollen texture identification using neural networks. – Grana 38: 59–64. ISSN 0017–3134.

The importance of research leading to the automation of pollen identification is briefly outlined. A new technique, neural network analysis, is briefly introduced, and then applied to the determination of light microscope images of pollen grains. The results are compared with some previously published statistical classifiers. Although both types of classifiers may work, the neural network is apparently superior to the statistical methods in three ways: high success rates (100% in this case), small number of samples needed for training, and simplicity of features.

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(*Manuscript accepted 31 May 1999*)

Pollen analysis is widely used in Quaternary and Pre-Quaternary palaeoecology and palaeoclimatic reconstruction and in palaeoenvironmental reconstruction for archaeology. It also has considerable commercial significance in the search for hydrocarbons and is of importance in medical studies. Yet pollen analysis is relatively undeveloped compared with, say, chemical analysis. It depends on the separate identification and counting of grains, mainly using human memory for comparison between fossil grains and reference taxa. These problems have limited the development of further links with ecology, and often failed to encourage the development of palaeo-population biology. It has been recognised for some time now that a potential solution to the problems of slowness and subjectivity will be automation (Flenley 1968, 1990). Only recently, however, has the idea begun to seem much more than a fantasy (Stillman & Flenley 1996, Green 1997). The present paper presents some rather promising results, suggesting that it may be not too long before automation, using pollen texture identification, is a practical possibility.

The importance of this new work is the possibility to achieve 100% accuracy using light microscope (LM) images. Previously, scanning electron microscope (SEM) images had been used, but these are expensive and difficult to produce for a mixed pollen assemblage. Although only a few pollen taxa were used, the present work shows a possible way towards the development of full automation of LM images of pollen grains.

Previous work on the automated identification of pollen taxa has been carried out by Langford (1988) and Treloar (1992). Both used SEM pollen images and statistical classifiers. Treloar's work is based on texture and shape analysis, Langford's on texture analysis only. For improving the classification rates Treloar explored artificial neural networks (Lippmann 1987, Hinton 1992) as classifiers. A 100% classification rate was achieved in three-taxon pollen identification (*Pritchardia minor*, *Passiflora quadrangularis*, and *Pseudoelephantopus spicata*). However, Treloar did not achieve a high classification rate in a four-class problem, which was to identify *Canthium barbatum*, *Macaranga graeffeana*, fungal spores, and *Xylosma suaveolens*. Afterwards the potential of using light microscope images was studied by Treloar (1994), and thirteen types of New Zealand pollen were analysed. Because of limited depth of focus (Stillman & Flenley 1996), the quality of LM images cannot be as high as that which comes from the SEM, and the classification rate achieved by the statistical technique was reduced.

However, recent research demonstrates that it is possible to use the neural network to improve the identification of pollen images obtained under LMs in three ways: using (1) simple variables (features) and (2) fewer patterns (samples), thereby achieving (3) 100% classification rate. In order to demonstrate this, three successfully trained neural networks are reported. The first network shows that based on the same data set it needs fewer training samples, and the second can clearly raise the rate achieved by the statistical classifiers. The last one needs only a simplified data set to reach the top rate. Therefore, the potential of using neural networks to improve the performance of classifiers working with LM images is demonstrated.

MATERIAL AND METHODS

Material

The taxa used in this study are four New Zealand pollen/spore types: *Hoheria populnea* (hereafter, HohPop; Fig. 1), *Phormium tenax* (PhoTen; Fig. 2), *Phymatosorus novaezelandiae* (PhyNov; Fig. 3), and *Podocarpus totara* (PodTot; Fig. 4). In such a small group of species the grains can be easily distinguished by their shapes. In this research, however, only texture analysis was used, as a test of its potential in automated palynology. There are 18 samples for each pollen species, and thus 72 texture images need to be identified.

Methods

The statistical classifiers used by Langford and Treloar are the well-known Fisher linear discriminant functions (Hand 1981). This

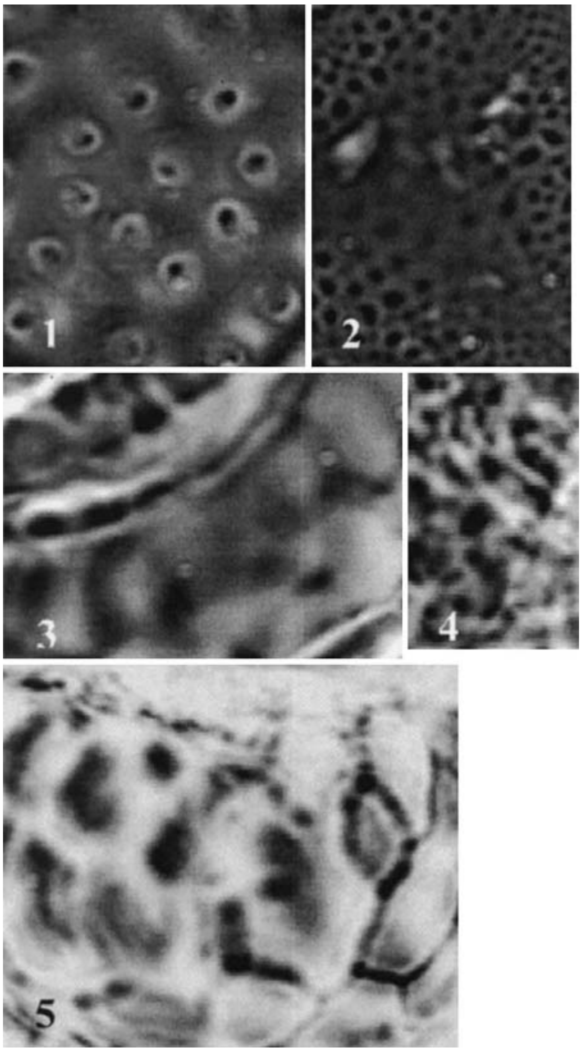

Fig. 1. Typical texture of *Hoheria populnea* (HohPop). *Fig. 2.* Typical texture of *Phormium tenax* (PhoTen). *Fig. 3.* Typical texture of *Phymatosorus novaezelandiae* (PhyNov). *Fig. 4.* Typical texture of *Podocarpus totara* (PodTot). *Fig. 5.* Texture of PhyNov16 which was misclassified as HohPop pollen.

technique is based on the hypothesis that a class of objects could be separated from another one by a hyperplane in a high dimensional space or a straight line in a two-dimensional space (i.e., a plane). In order to build such a space, necessary variables must be selected from a possible candidate set. Obviously, when more candidate variables are available, there are greater chances of having the required space. To calculate the classification rate a leave-one-out technique was employed (Langford 1988, Treloar 1992, 1994). That approach is often used when the classifier is constructed from a small set of samples. In that case, reservation of some samples for testing the classification rate will heavily influence the successful training of classifiers. Therefore, each time only one sample is reserved for the test set, and the rest become a training set. Thus, each classifier is trained by more samples and each sample has a chance to do the test. However, it is necessary to construct as many classifiers as the samples. In this study, we find that only part of the 72 pollen texture patterns is enough to train a neural network that can successfully assign the remaining patterns into the four pollen types. Therefore, the leave-one-out method is not adopted, which greatly reduces the computation required.

The neural network used is a feedforward type of Multi-Layer Perceptron (MLP, see e.g. Rumelhart et al. 1986, Beale & Jackson 1990, Weiss & Kulikowski 1991). Three types of neurons or units are organised in the layers, and there are connections between two adjoined layers (Fig. 6). The units for directly presenting features or variables into the units in the next layer through connections form an input layer. An output layer consists of output units each representing a category in the classification task. Between the input and output layers in the MLP, there is at least one layer of units. They are usually named hidden layer and hidden units, respectively, as they associate indirectly with the real world. The hidden unit has a non-linear transfer function (Wasserman 1989), and the MLP as well as the classifier it implements is thus non-linear in nature. The function in the output units may or may not be non-linear, depending on the applications of MLPs. If the MLP serves as a classifier, the functions should be non-linear. The non-linear nature plays an important role in neural networks, but no non-linear transformation happens in input units. Thus, some researchers take into account only the hidden and the output layers when they number the layers in the network. For instance, they call the MLP shown in Fig. 6 a three-layered MLP, while we consider it to have four layers. The structure of this network can be denoted as 5–4–4–3. Data for description of features or variables presented by the input layer go through the hidden layer(s), and arrive in the output layer to make decisions. Since there is no information propagated in reverse, this type is known as the feedforward neural network.

After the topological structure of the MLP is chosen, values known as the weights represented by the connections must be determined. A training algorithm, called error back propagation (Rumelhart et al. 1986), is used to adjust the weights in order to make the actual output satisfactorily match the desired one. The training phase is stopped when an acceptable match is met. Then patterns that the network has never seen during its training are used to test the trained neural network classifier. The classification rate can be defined as a ratio between the number of correctly classified patterns and the number of whole patterns in the test.

The pattern consists of input data and desired output data. The input data that Treloar (1992, 1994) employed were five commonly used Haralick texture measures, (i.e., Angular Second Moment, Contrast, Entropy, Inverse Difference Moment, and Variance; see, Haralick et al. 1973), ten Laws masks (3×3 and 5×5; see, Laws 1980, Pietikäinen et al. 1983), and six frequency matrix measures (Shen 1982). In order to cope with the relatively poor LM images

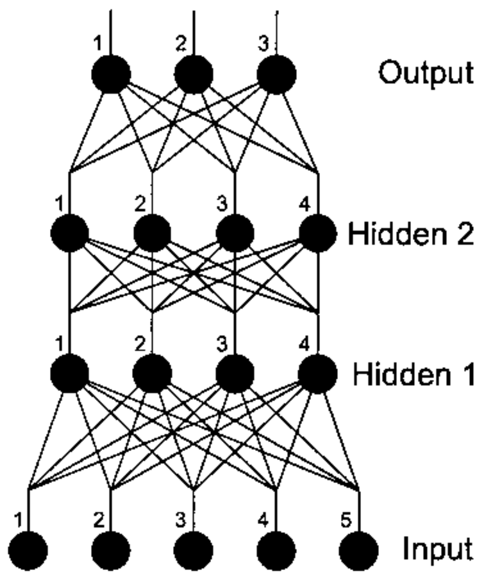

Fig. 6. Structure of a 5–4–4–3 feedforward multi-layer perceptron neural network.

Treloar (1992, 1994) increased the number of variables. Some data processing techniques were adopted, including Sobel convolution and root-of-two modifier (Langford 1988). Thus, Treloar used four types of Haralick measures: a standard one (Type 1) and three variants as shown in Table I. Moreover, the displacement in the co-occurrence matrix calculation (Langford et al. 1990) was extended from 1 to 32 pixels with an increment of one pixel (Treloar 1994). Consequently, there were 640 Haralick variables: 5 Haralick texture measures times 4 types, and then times 32 increments. In addition, there were 20 Laws masks, and 72 frequency matrix measures. Therefore, there were altogether 732 variables available in Treloar's texture data set.

The desired output represents the class to which the corresponding patterns are assigned. A binary number 1 marks the class that the pattern is in, while binary 0 indicates the class that the pattern is not in. Therefore, the desired output should consist of one 1 and several 0's. It indicates misclassification if either actual output from the classifier is all 0's, or it has more 1's, or the unique 1 is assigned to another class.

RESULTS

Neural network classifiers MLP1 and MLP2 based on the data sets used by statistical classifiers

The four-pollen-taxon problem was solved based on eight selected variables, and a 100% classification rate was achieved by leave-one-out classifiers (Treloar 1994, Treloar & Flenley 1996). All the selected variables were Haralick texture measures. They were the type 1 of Angular Second Moments with displacement of 1, 2, 3, and 4 pixels, the type 2 of Angular Second Moments with displacement of 2, 3, and 6 pixels, and the type 2 of Contrast with 9-pixel displacement. Based on these eight texture measures an 8–3–4 neural network MLP1 was successfully developed for the same data that Treloar (1994) used. Of these, 28 pollen samples are employed for training the network, and 44 samples are reserved as the test data (Table II). The test shows that the 100% classification rate is also achieved by the MLP1. Unfortunately, the same statistical classifiers are not reproduced because the ability of the variable selection is constrained by the power of the program written by Treloar using the software package of Borland Pascal version 6. Although he prepared 732 texture variables, the program can only select variables from a subset of 16 candidates at a time. The next selection is performed on a new subset that consists of already selected variables and other candidates, which have not been optimised. It does not stop until all the candidates have been checked. Thus, the optimised result depends on the initial subset and the order of the candidates in the queue. Effort is spent on reproduction of the same aforementioned eight variables from all the four types of Haralick texture measures. It fails because the order of variables in Treloar's study is unknown. Moreover, the optimisation is designed as an indispensable step in building the classifier. Thus, when Treloar's eight variables are used to reproduce the classifier, only seven out of the eight variables can pass the optimisation to participate in the establishment of classifiers, while another variable is never acceptable. The denied variable is the type 2 of Contrast, and the classification rate for the leave-one-out statistical classifiers is not completely 100%. A confusion matrix is given in Table III to show the percentage rate. The matrix reveals that one out of 18 PhyNov samples is confused with the pollen HohPop ($1/18 = 0.0556$). This misclassified sample is PhyNov16, and Fig. 5 shows that it seems more like HohPop than other typical PhyNov samples.

Since the statistical classifiers based on seven variables cannot completely identify all the four types of pollen, a neural network classifier, MLP2, is developed as an alternative. The structure of it is 7–3–4. Based on the same seven variables, the classification rate that MLP2 achieves is completely 100%, and thus no confusion matrix is required at all. Moreover, MPL2, as well as MLP1, is also superior to the statistical classifiers reported in Treloar (1994) and Treloar and Flenley (1996). This is because the training set is smaller than those used in developing the leave-one-out classifiers, while more samples are reserved as test patterns. For details of the training and test sets, see Table IV.

Table III. *Classification rate (%) given by leave-one-outstatistical classifiers based on the seven selected variables.*

Desired	Class Matched			
Class	HohPop	PhoTen	PhyNov	PodTot
HohPop	100	0	0	0
PhoTen	0	100	0	0
PhyNov	5.56	0	94.44	0
PodTot	0	0	0	100

Table I. *Four types of Haralick variables used by Treloar (1994).*

1	2	3	4
Without $\sqrt{2}$ Operation		With $\sqrt{2}$ Operation	
Without Sobel Operation	With Sobel Operation	Without Sobel Operation	With Sobel Operation

Table II. *Number of samples used in the training and test sets of MLP1.*

Taxon	Training	Test	Sum
HohPop	6	12	18
PhoTen	6	12	18
PhyNov	8	10	18
PodTot	8	10	18
Sum	28	44	72

The success of MLP1 and MLP2 shows the potential of neural networks in the identification of pollen grains from their texture images produced from light microscopes. It also illustrates that the 72 pollen samples are adequate for MLP development. Thus, it seems that all the variables adopted by Treloar are not necessary and could be simplified. This forms the topic considered in the discussion.

Based on the commonly used five Haralick texture meas-

Table IV. *Number of samples used in the training and test sets of MLP2.*

Taxon	Training	Test	Sum
HohPop	6	12	18
PhoTen	5	13	18
PhyNov	6	12	18
PodTot	9	9	18
Sum	26	46	72

ures, a simple neural network, MLP3, is developed with 5–2–4 structure. Its input and hidden layers have fewer units than MLP1 and MLP2.

The successful training is based on 21 samples, and the remaining 51 samples are completely identified in the test phase, i.e., the classification rate is 100%. The decreased training data set and the pattern-increased test set are given in Table V.

DISCUSSION

Comparison of the statistics and neural network methods

Since MLP1 and MLP2 show that neural network classifiers using Haralick texture measures can cope with the LM images, the Laws masks and frequency matrix measures are not used in this stage of the study. Because an early study had shown that the displacement in the Haralick measure calculation could be limited to just two pixels (Weszka et al. 1976, Conners & Harlow 1980), we simply use one-pixel displacement. Thus, the $\sqrt{2}$ modifier does not work. In addition, the Sobel operation is designed to enhance boundaries of regions in the image to benefit human vision. It may not be necessary in computer vision, and thus could also be disregarded. It means that the commonly used Haralick texture measures are adequate for neural networks to handle the four-taxon-pollen problem. Since most variables in Treloar's data set can be neglected, the computation for variable selection, necessary in the development of statistical classifiers, (Treloar 1992, Stillman & Flenley 1996) can be ignored.

Of the five Haralick texture measures only the Angular Second Moment was selected as a necessary variable to build the statistical classifiers discussed in the results section. Since the optimisation program does not ensure an optimum selection, it is probable that the five variables could lead to a satisfactory classification. Thus, they are used to establish the leave-one-out classifiers for comparison with MLP3. Unfortunately, the statistical classifiers obtained almost fail to recognise two pollen types. Their confusion matrix is given in Table VI.

Table V. *Number of samples used in the training and test sets of MLP3.*

Taxon	Training	Test	Sum
HohPop	4	14	18
PhoTen	5	13	18
PhyNov	7	11	18
PodTot	5	13	18
Sum	21	51	72

MLP3 brings another superior property: it is possible to visualise the separation of different pollen taxa on a plane as MLP3 has only two hidden units. Outputs from the hidden units can be considered as attributes. Although they are not presented directly into the neural network classifier, they are direct inputs to make decisions in the output layer. The hidden units produce the mappings of the original patterns, dimensions of which are reduced from five to two. From the output of the hidden units to the output layer, it is a traditional single layer perceptron (Minsky & Papert 1969), the infancy of the modern MLP. The perceptron shows that it is possible to use only two attributes to identify the four pollen taxa as long as a good input representation combined with sufficient examples is given. This can be illustrated in Figs. 7–9.

The scatter diagram (Fig. 7) shows that the HohPop samples are mapped in the top right corner of the two-dimensional space, and the PodTot ones are mapped in the bottom left corner. According to these two new attributes (x_1 and x_2), it is very easy to find straight lines to separate one class of the mappings from another in the two-dimensional space (Li & Flenley 1997). This is exactly what the Fisher linear discriminant function tries to do. The sample of PhyNov16, misclassified into the HohPop type by the statistical classifier mentioned in the last section, is now mapped far from the HohPop region and thus ensures the success of MLP3.

CONCLUSIONS AND FUTURE DEVELOPMENTS

Three feedforward neural network classifiers are presented to show the potential for identification of pollen texture images obtained under a light microscope. A comparison with statistical leave-one-out classifiers based on the Fisher linear discriminant function has been carried out. Although the two types of classifiers may be successful based on different texture features, neural networks can give superior identification and need fewer samples with fewer and simpler features. Three MLPs demonstrate that neural networks are also suitable for pollen identification, and may have an important part to play in the ultimate automation of palynology.

The MLP method will need to be expanded and developed to include a larger number of pollen types to meet requirements of palynology. Therefore, the next work will apply this technique to identify other pollen groups that consist of six, seven, and ten New Zealand types of pollen, respectively.

Table VI. *Classification rate (%) given by leave-one-out statistical classifiers based on five type 1 variables.*

Desired Class	Class Matched			
	HohPop	PhoTen	PhyNov	PodTot
HohPop	70.37	14.81	14.81	0
PhoTen	0	37.04	20.37	42.59
PhyNov	0	22.22	77.78	0
PodTot	0	48.15	3.70	48.15

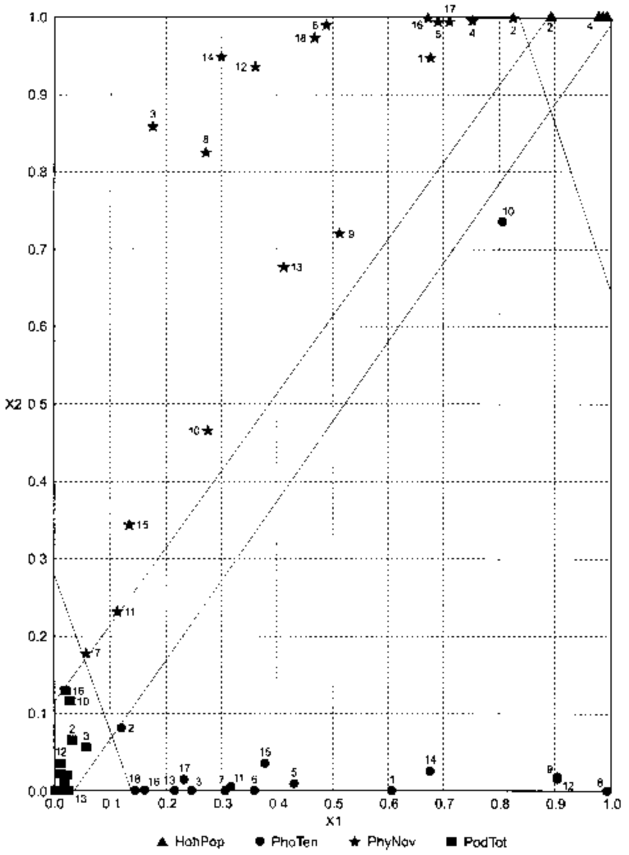

Fig. 7. Separation of four pollen taxa in a 5–2–4 neural network, MLP3. Four straight lines show the boundaries of the four types of pollen mapped by MLP3. PhyNov 16 was misclassified in to HohPop by a statistical classifier and is now mapped far from HohPop by MLP 3. This scatter diagram is a two-dimensional mapping from five dimensional texture measures. For the detailed distribution of most HohPop see Fig. 8. For the detailed distribution of PodTot see Fig. 9.

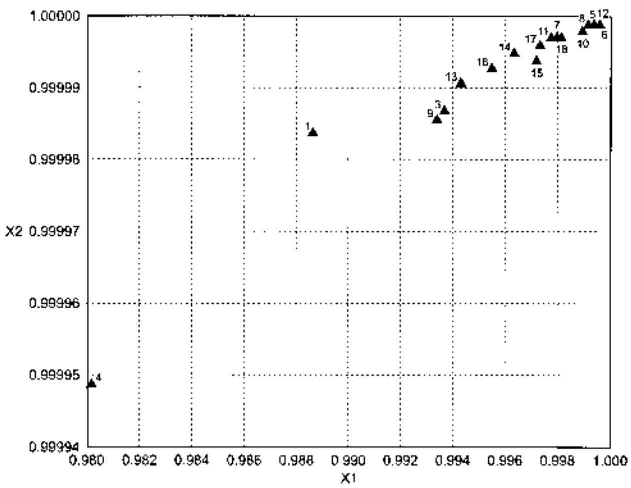

Fig. 8. Enlargement of the area for mapping most of the HohPop samples.

Obviously, to identify additional types and/or pollen grains, more variables or more effective types of variables may be needed. Because the numbers of units in the input and output

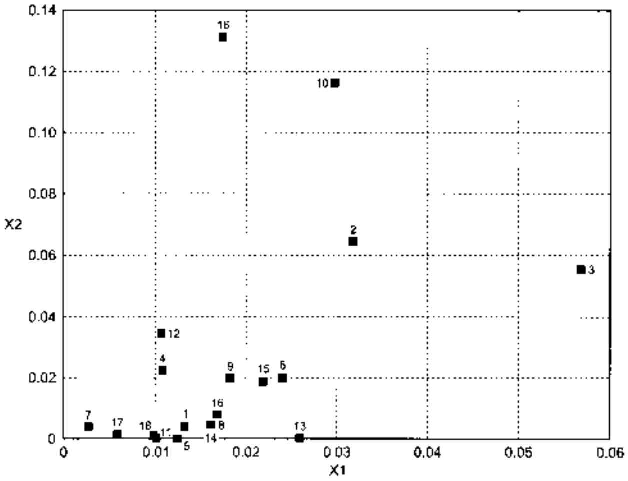

Fig. 9. Enlargement of the area for mapping of PodTot samples.

layers depend on a specific task, neither additional variables nor additional types of pollen for an expanded task are acceptable. The structure of the neural network has thus to be designed for the different task. Also, to train a more complex network will consume a longer time than before. This is because personal computers (PC) have to simulate the neural network. The original idea of neural networks was that massive processing units were connected as a network and ran in parallel. Therefore, the structure does not affect the speed significantly when the network processes information. However, the PC has one processing unit so it has to deal with neural units one by one.

The Sobel operation was used by Treloar (1994) to enhance the boundary to distinguish pollen grains from their environments. On the other hand, neural networks perform nonlinear transformations that can be more powerful than the Sobel operation and other similar image boundary enhancement techniques. Therefore, pre-processing of boundary enhancement may not be necessary to the neural network. The advantage of neural networks shown in this paper is that they do not need such pre-processing and we do not need to create massive candidate variables and then to optimise them one by one. Since our networks can identify pollen grains without any enhanced boundaries, we may not adopt boundary enhancement pre-processing in the future developments.

ACKNOWLEDGEMENT

This project is supported by a Massey University Postdoctoral Fellowship for one of us (P. Li). The authors would like to thank Dr. W. Treloar for the pollen LM images and his programs on statistical classifiers, and our colleague Leighanne K. Empson for her valuable assistance. We would also like to thank Karen Puklowski for redrawing some of the diagrams. Thanks are also due to Dr. E. C. Stillman, Mr. A. E. Karaman and Prof. H. J. B. Birks for their helpful comments.

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