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A new approach to automated pollen analysis

I. France[a], A.W.G. Duller[a,*], G.A.T. Duller[b], H.F. Lamb[b]

[a]School of Electronic Engineering and Computer Systems, University of Wales, Bangor, Dean St., Bangor, Gwynedd, LL57 1UT, UK

[b]Institute of Geography and Earth Sciences, University of Wales, Aberystwyth, Ceredigion, SY23 3DB, UK

Abstract

Palynological data are used in a wide range of applications, but the tasks of classification and counting of pollen grains are highly skilled and laborious. The development of an automated system for pollen identification and classification would be of great benefit. Previous attempts at computer classification have taken approaches that have been intrinsically difficult to develop into fully automated systems that could operate largely independently of a human operator. We describe a new approach to the problem based on improving the quality of the image processing rather than the data collected using images collected with an optical microscope. Two sets of experiments are described, demonstrating the ability of the system firstly, to differentiate between pollen and detritus, and secondly, to classify different pollen types correctly. The results of these tests, in which the pollen images were acquired using an automated system, are encouraging and demonstrate that even using relatively low spatial resolution we can reliably differentiate between three taxa of pollen grains. Based upon the experience that we have gained we describe the characteristics required of the next generation of automated pollen identification and classification systems.

1. Introduction

Fossil pollen spectra are widely used for palaeoenvironmental reconstruction, both within the Quaternary and further into the geological past. Sites which preserve a continuous sedimentary record, such as lake basins e.g. Tenaghi Philippon (Tzedakis et al., 1997), provide long pollen time series that can be interpreted in terms of environmental change. The abundances of different species are plotted as a function of depth or time, expressed in terms of the total abundance of pollen, the percentage of each type of pollen within a horizon, or as a proportion relative to an exotic species that has been added to allow the calculation of absolute pollen fluxes. Subsequent analysis of these diagrams is normally qualitative, though transfer functions are increasingly being used to obtain quantitative values for specific palaeoclimatic parameters (e.g. Huntley, 1993).

Pollen analysis is time consuming, laborious and highly skilled work. Standard measurements require the classification of at least 300–500 pollen grains from each slide. Additional counting is required if a slide is dominated by a few taxa, or if numerical estimation of the concentration of rare grains is required. The use of numerical transfer functions to calculate palaeoclimatic parameters will place increasingly high demands on the quality of the data obtained. Green (1997) considered the "sheer time and effort involved in counting pollen" as one of the main hindrances to the wider application and development of the technique. Stillman and Flenley (1996) summarised the advantages that would arise if an automated pollen identification and classification system could be developed. In brief, these include a decrease in the time and cost of analysis, a consequent ability to increase the sampling resolution used, being able to increase the number of grains that are routinely counted, and complete objectivity in identification. However, in spite of several previous attempts, no routinely applicable system has been successfully developed to date.

1.1. Previous attempts to automate pollen analysis

A number of attempts have been made to develop computer tools to help in the classification of pollen types. Many computer-aided systems have been developed in the past that were little more than automated classification keys (e.g. Guppy et al., 1973; Straka, 1991) or databases of pollen images. While this type of tool is clearly of great value, it is not a truly automated

* Corresponding author. Tel.: 0044-1248-382-686; fax: 0044-1248-361-429.

E-mail address: duller@sees.bangor.ac.uk (A.W.G. Duller).

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procedure, and is quite different from the aim of the work described here.

Langford et al. (1990) developed a computer system whose purpose was the classification of pollen grains based on analysis of their surface texture. The system that they presented was based on the use of scanning electron microscope images of grain surfaces. The images were deliberately chosen so that they were from areas of the grain surface that excluded any "edges, apertures, folds or other anomalies" (Langford et al., 1990). The surface textures of 32 pollen grains of six different taxa were measured, and used to construct a classification system based on a subset of 15 different measures of the texture. A very efficient classification could be achieved, with over 90% of the grains correctly identified, using a combination of just two measures of texture. The technique was successful, but would be difficult to fully automate because it would require the preparation of a whole sample for SEM analysis, and it still requires a human operator to select suitable parts of each grain for analysis.

Li and Flenley (1997) have described initial work on texture analysis using light microscope images and given results for four New Zealand pollen types. Li et al. (1998) discuss an extension to the previous paper and describe the use of a variety of multi-layer perceptron (MLP) neural network configurations for the separation of 13 types of New Zealand pollen. Their approach is based on a set of texture measures which are specific to each pollen group being analysed. While the results of this approach were impressive for the data set concerned it is clear that it will require a considerable amount of effort to extend the work, since the textural measures would have to be altered or new measures added whenever a new pollen taxon was added.

1.2. Requirements for an automated pollen analysis system

What is noteworthy about all of the previous attempts to automate pollen recognition is that none of them were potentially capable of producing a truly automated system since none of them addressed the fundamental problem of locating a pollen grain mounted on a slide. While this is a simple matter for a human operator, this is not trivial for a computer-controlled system, yet is essential if a system is expected to identify a large number of grains from each slide. In this paper we differentiate between two important tasks for an automated pollen analysis system. Pollen identification, as defined here, is the task of finding the location of a pollen grain in an image or series of images taken from a slide. It involves differentiation between pollen grains and a range of detrital materials found on a slide. The second task, which is wholly separate, is the classification of the pollen grains identified in the first step into different taxonomic categories. The terms identification and classification are consistently used for these two steps throughout this paper.

A truly automated pollen analysis system should be able to operate without requiring specialised preparation procedures beyond those currently in use. Additionally, it would be advantageous if as little specialist equipment as possible were used so that such systems could be set up by many laboratories. Therefore, the system should consist of a standard optical microscope equipped with a computer-controlled stage, a digital imaging system and a computer to control the movement of the slide under the imaging system and to undertake the analysis of the objects that are found.

A number of levels of sophistication of such a system can be imagined. At the lowest level the system would simply scan a slide, identifying the position of pollen grains, and rejecting any detritus. As mentioned above, this has never been achieved before, but is an essential prerequisite for any automated system. At this level of sophistication the system would then present a series of digitised images to the user who would then classify them manually. In an enhanced version, the operator would be able to alter the resolution of the images that were displayed, and if necessary ask the computer to return the stage to the location of a particular grain. This system would substantially reduce the time spent by an operator in searching for pollen on the slide, and would be of particular value where slides were very sparse, or the amount of debris high.

The next level of sophistication would involve the computer classifying the objects that are found into a number of categories. For a simple system the classification may only be able to identify a proportion of the pollen grains observed (perhaps the most common species), and the remainder would still require identification by a human operator in the same procedure as described above.

The ultimate level of sophistication would be a system that was able to identify and classify the vast majority of pollen grains on a slide and produce absolute numerical data at the end of the analysis. In such a system the operator would only be required to act in a supervisory role, confirming the classification of any ambiguous grains, and reviewing complete system performance.

This paper describes recent work that has utilised a novel method for automating pollen analysis based on a neural network designed specifically for image processing. A number of other image processing techniques are also used where appropriate. This method can potentially overcome many problems highlighted above, and could in theory lead to the development of an entirely automated procedure. The work presented here includes some initial tests of the approach, in order to show first that the system can locate pollen grains on a slide and differentiate these from detrital material, and secondly that different types of pollen can be classified reliably in a controlled test.

2. Characteristics of neural networks

The major alternative to neural network approaches are those known as "model-based". These methods require the a priori construction of a detailed description of each taxon by the operator. Analysis of an object is conducted by comparison with these models. The large morphological variations that occur within a single pollen taxon due to deformation or viewing angle are such that standard model-based approaches are unlikely to be successful. The approach taken in this work to pollen recognition is one based on a neural network. A neural network is a system that can be trained to classify input data (in our case images of pollen grains) into one of a number of classes. Neural networks are particularly well suited to this type of application because they do not require the individual setting up the system to define key characteristics of the objects. Instead the system creates its own internal representation of the objects based on a training data set.

2.1. The Paradise neural network

The neural network used here is known as Pattern Recognition Architecture for Deformation Invariant Shape Encoding (Paradise) and was designed for the recognition of visual objects. It has been applied to a number of recognition tasks and has demonstrated its ability to classify hand gestures (Banarse and Duller, 1996), faces and handwritten numerals. The feature recognition network (FRN) developed by Hussain and Kabuka (1994) provided the inspiration for this network, giving deformation tolerance while keeping the network small and practical. Unlike the FRN, however, the Paradise network is able to work with grey-scale images and can be trained on or off line using unsupervised learning. In practice, this has three effects. Firstly, no pre-processing of the input data is necessary to produce a binary image, though other pre-processing may be beneficial to reduce noise or remove artifacts. Secondly, the ability to learn on-line means that there is no need for a highly iterative learning phase prior to the network being usable for recognition, and new training data can be added at any point in the life of the network. This is in contrast to neural networks such as the Multi-layer Perceptron which have to be trained on a set of data (often a very long process) and once trained they can be used for recognition. If there is a requirement to add more training data then the learning must be performed again on the entire training set. The third effect is that the training data set does not have to be labelled prior to training. With supervised learning, as used by the Multi-layer Perceptron, the training data must consist of the input data together with the output that is to be expected.

Paradise uses a method based on creating small templates (PDMs) pattern detection modules which are responsible for identifying the important features of an object. The identification is then made on the basis of linking several of these templates together in a classification layer. The network has a three-layer architecture:

- The feature extraction (FE) layer.
- The pattern detection (PD) layer.
- The classification (C) layer.

A schematic representation of the network is shown in Fig. 1. The input to the network is an image of a single pollen grain. The network then assigns the input image to an output class. The number of classes that will be created is not predetermined but is dependent on the data and the similarity criterion ("classification threshold") used to place objects in the same class. By varying the "classification threshold" different network behaviour can be obtained. For example, with a low threshold only a gross separation of the objects will be achieved into a small number of classes. By increasing the threshold a finer separation can be produced at the expense of increased computation time.

2.2. The feature extraction (FE) layer

The FE layer consists of a single layer of FE planes. Each plane extracts a certain type of feature from the input image. For example an FE plane could find all of the horizontal edges that occur over a distance of five pixels. In this particular application FE planes were used which extract horizontal and vertical edges at various frequencies, an example of the output of a pair of FE planes for one input pollen image are shown on the right of Fig. 1. The particular frequencies were chosen by examining the lines extracted from pollen images at a number of frequencies and choosing those that generated the most information.

2.3. The pattern detection layer

This layer builds up a set of templates, or pattern detection modules (PDMs), from the features produced in the FE layer. Each PDM is responsible for recognising a single template made up of features taken from each of the FE planes (Fig. 1). Due to the relatively small size of these templates, they can often be reused to represent parts of many objects. The templates are not preset by the user or by the system but are generated automatically during training. When PDMs are attempting to recognise features in an image the position of the template is allowed to move in a constrained manner to allow tolerance to deformation and small translations. Thus the image being recognised does not have to be identical to

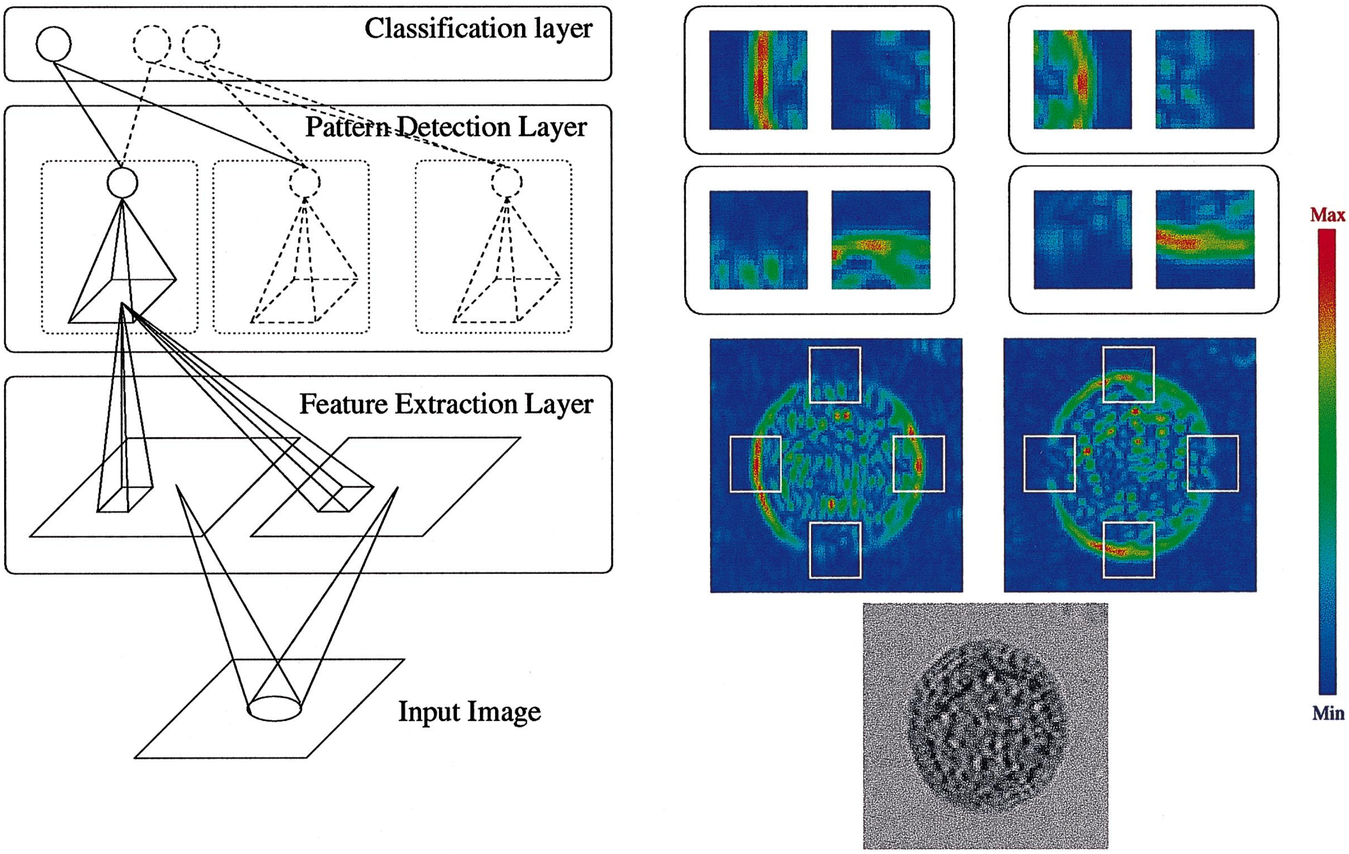

Fig. 1. Schematic of the Paradise neural network, showing the way in which the initial image of a pollen grain is analysed through the 'Feature Extraction Layer', the 'Pattern Detection Layer' and finally the 'Classification Layer'. On the right of the diagram are examples of the data analysed at each level. At the bottom is the initial image collected from the microscope. The next level shows the operation of the Feature Extraction Layer in highlighting horizontal and vertical elements. The upper images in the four ellipses are examples of pattern detection modules generated by the network. Each PDM corresponds to a small part of the total image.

the stored templates but is allowed to vary a certain amount which is determined by a user-chosen parameter.

2.4. The classification layer

Having created the templates the object is represented (classified) using a number of the templates, this information being encoded using links to a classification cell in the classification layer. Thus a model of the important aspects of the input image is encoded using a collection of PDMs and a single classification cell is used to represent this class of objects. When subsequent objects are presented to the network the existing classes are examined to see if a sufficiently good representation already exists, if not a new class is created together with any new PDMs which are required to represent the new object. Thus the network "grows" as images are presented to the network.

2.5. Neural network parameters

There are a number of parameters which can be set to control the type of recognition which is performed by the network. The majority can be set using heuristics and generally stay fixed for a given application. Once these are set the "classification threshold" parameter is used to determine the degree of match that is required between the input object and the internal template model. Whilst the classification threshold greatly affects the response of the network, Banarse and Duller (1997) have shown that in terms of the qualitative results it is only important to get the value in the "right area", i.e. the changes in network behaviour are gradual with changing threshold.

3. A practical automated pollen analysis system

The work presented in this paper forms part of a pollen identification and classification system. In this section we outline how such a system would be used in practice. Since we are using neural networks as part of the recognition system there will need to be a training phase in which images of pollen of known type are presented to the system. This is usually known as the learning or training phase of neural network operation. When an unseen pollen image is presented to the network an output class will be identified which in turn identifies the pollen. It is important to note that with the type of neural network being used here there will usually be more than one class representing a single type of pollen. This is because the same type of pollen appears very differently depending upon its orientation, or degree of degradation. An essential part of the training phase is for the operator to tell the system which of the classes it has created are representative of a given pollen type. In general, there is no problem with a single type of pollen being represented by more than one class, so long as each class contains pollen of only one type.

Once the system has been trained, it can be presented with unknown images and the output of the network is the class that most closely represents that image. This is often referred to as the recall phase.

3.1. Hierarchical processing of pollen images

Images collected by the CCD camera go through a hierarchy of processing operations within the software (Fig. 2). The initial images that are acquired may contain one or more pollen grains and variable amounts of debris. The aim of the processing system is firstly to identify the position of any pollen grains (stages 1–3), and secondly, to classify those pollen grains into various groups (stage 4).

This hierarchical approach has been adopted because the resolution of the image data required at each stage varies considerably. For example, a much lower spatial resolution is required in order to differentiate between pollen and non-pollen objects than for pollen classification. Given that the processing time increases as at least the square of the resolution, a hierarchical analysis structure allows the system to be optimised by selecting appropriate resolutions of data. The following sections describe in detail the four stages involved in identification and classification of pollen grains.

3.1.1. Stage 1

Initial processing of the image determines the positions of any possible objects, be they debris or pollen grains, within the image. The images obtained from the microscope can contain several objects. The following process is used to extract these objects from the image.

Before starting an automated scan over the slide a reference image is obtained of a blank section of the slide. This is used to correct for uneven illumination across the field of view by subtracting it from the images obtained during the scan. After correction for the variation in the background images taken across the slide are filtered with a 7×7 variance filter. This gives a measure of how much variation is occurring in the image intensities around each pixel. The background tends to give low variance (since it is fairly featureless) and objects give a high variance. In order to detect the foreground and background pixel automatically a k-means algorithm is used to partition the variance image into two sets. The set corresponding to the lowest variance is taken as background. This image is smoothed to remove single isolated

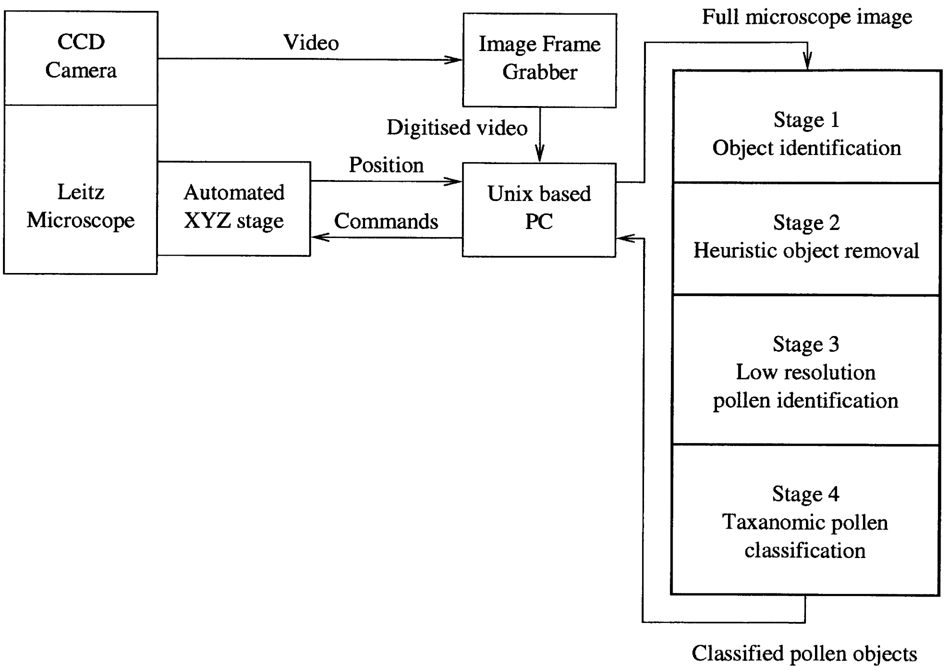

Fig. 2. Block diagram of the automated pollen analysis system.

pixels and close up some holes within the objects. To extract the objects, pixels within the image that are connected to each other are linked to form groups of connected components. These components and the area around them are then extracted from the image and passed to the next stage to start the process of differentiating between pollen and debris.

3.1.2. Stage 2

After an object has been found in the image it is checked against four size criteria. The number of pixels within the object must lie within certain limits, and its bounding box must be less than a given width and height. These heuristic criteria rule out many small objects such as flecks of dust, and eliminate larger objects such as pieces of charcoal or detrital plant material. The values for these thresholds must be chosen bearing in mind the size of the pollen being searched. In general, these limits are set loosely in order to prevent pollen from being rejected incorrectly. The third stage is designed to remove any remaining debris.

3.1.3. Stage 3

In the third stage objects passed by the second stage are presented to the first Paradise neural network. This network is designed to be fast and produces only a coarse separation between pollen and other objects. In order to increase the speed it analyses the images at lower resolution than the original images (Fig. 3). In our system the image resolution is halved along each of the x- and y-axis, resulting in an image containing one quarter of the information of the original. This data loss means that there is not enough information to properly classify pollen types, but enough remains to allow the further removal of debris from the set of images.

The images collected from Stage 2 are presented to the network and the network is trained on them. The network will separate the images into classes of similar shapes. A human operator then examines the sets of images associated with each class and decides whether the class represents pollen or debris. As described by France, Duller et al. (1997) this trained network can then be used to separate new pollen and debris images automatically. Any images which are classed in pollen classes are presented to stage 4.

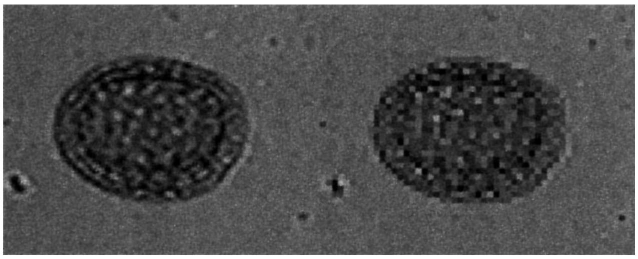

Fig. 3. An example of the two image resolutions used within the system. The image on the left is the full-resolution image used for object identification, while that on the left is the reduced resolution used to differentiate between pollen and non-pollen objects.

3.1.4. Stage 4

The fourth stage involves a second neural network that classifies the pollen images into different taxonomic groups. In the training phase separate Paradise networks are trained to recognise individual pollen types based upon their analysis of reference pollen slides. These recognisers are then combined, as required, into a larger network that classifies the different types. This combined network tests each input image against all the classes from the separate networks and assigns the image to the class to which it is most similar. If an image is not sufficiently similar to any of the classes it is rejected by the network.

The network assigns each object that is presented to it into one of the classes. In the training phase the Paradise network automatically generates its classes when an unfamiliar object is presented to it. In order to make a "real world" classification the network must be told which Paradise classes are associated with each pollen type. This training can be performed at presentation time or after the network has been trained on a large number of images. The second approach is preferable as an operator can view all the objects associated with a class and make a swift judgement as to the nature of the class. With the first method the operator must identify each object individually; a time-consuming task. However, this would allow the network to perform an analysis of the relationship between the Paradise classes and the "real world" classes and is useful for testing purposes. In general, there is no problem with a single type of pollen being represented by more than one class within the neural network, so long as each class contains pollen of only one type. Any classes containing mixes of different types of pollen could be subjected to further examination using an additional Paradise network with greater discriminating power.

4. Results

The results presented here have been obtained using a Leitz Ergolux AMC trinocular microscope equipped with a Quick Step computer controlled X-Y-Z stage. In all other respects the optical system is a standard microscope as would be used by a human operator undertaking pollen identification.

In place of a still camera, the system utilised a standard monochrome CCD camera, with a resolution of 768×576 pixels, attached to a frame grabber capable of digitising the image with up to 256 grey levels. All the images have been obtained working at a magnification of

250 times. The combination of the X-Y-Z control of the stage and the CCD camera allows complete computer control of the processes involved in obtaining a series of images of the slide. Computer processing of the results described here was undertaken using a Pentium II 266 MHz PC running UNIX (Fig. 2).

Pollen residues prepared using standard techniques were mounted on glass slides in the conventional manner. Two different mounting media were tested. Initial tests were undertaken using glycerine gel. As discussed later, variation in the focal plane in which the pollen grains lie is a major problem in this system, and in an attempt to reduce this problem, later measurements were made on slides prepared with ClearCol. While this did appear to reduce the variation in the vertical position of the grains, it was still not possible to assume that all grains lie in the same plane.

Sets of images were collected from three reference pollen slides. The pollen types used were *Polemonium caeruleum*, *Nymphaea alba* and *Crataegus monogyna*. Stage 1 (collection) and stage 2 (size filtering) are intimately linked, and take approximately 30 s to split a full-size image into images of individual objects. Examples of the objects collected by the first two stages are shown in Fig. 4. The numbers of images left after the image sets have been presented to stages 2 and 3 are shown in Table 1. The stage 3 processing has removed large amounts of debris from the image sets, including some badly deformed pollen and several examples of clusters of pollen. Currently, the stage 4 classification process is not able to deal with severe deformations or clustering. The processing times for stage 3 were 5.3, 48 and 1.9 min for the *Polemonium*, *Nymphaea* and *Crataegus*, respectively. The increased time for the *Nymphaea* is due to the large amount of images collected from the slide that contained large amounts of detritus.

As described above for Stage 4 processing the sets of images from stage 3 were used to create three recognition networks. These were then combined to create a classification network for the three pollen types. The results of randomly presenting the images from all the pollen sets to this combined network are shown in Table 2. Note the addition of a reject column to this table. Once trained the network will reject any input image that is not sufficiently close to the classes it has learnt. Some of these rejects are in fact badly deformed examples of pollen grains but most are not; they are just very different from the classified images and each other. The amount of variability in pollen grains cannot really be represented accurately with the small data sets being used here. As the number of training images increases the chances that pollen grains rejected by the current network are sufficiently similar to new grains to be incorporated into a network class also increases and so the percentage of rejected images should decrease.

The network classes created in stage 4 and the images classified by them are shown in Fig. 5. Each row of this image corresponds to a single class. The numbers on the far left give the class identity and the number of objects recognised by that class. The images within the rows are arranged in descending order of recognition quality. The times for training each of the classes (which only need be done once) were 166, 190 and 88 min respectively. The recall stage involving the recognition of all 204 objects took 121 min.

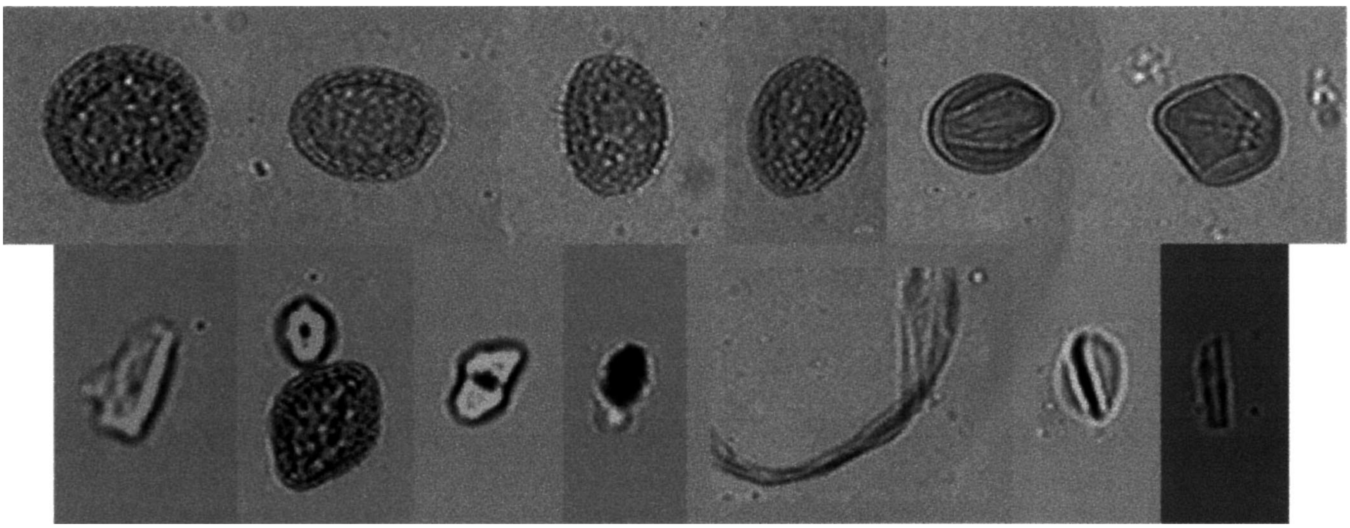

Fig. 4. Examples of the two classes of images collected at the end of stages 1 and 2, as described in Fig. 2. In these cases, the upper set of images were classified as pollen, while those below were not. In one of the images that was rejected, a pollen grain can be seen, but because it is overlain partially by a piece of debris, it was not identified as pollen.

Table 1
Number of images remaining after different stages in the removal of debris objects

Pollen type	Number of images remaining	
	After stage 2	After stage 3
Polemonium	138	60
Nymphaea	467	60
Crataegus	117	84

Table 2
Results from the classification neural network in stage 4

	Recognised as...			
Actual Type	Polemonium	Nymphaea	Crataegus	Rejected
Polemonium	47(78%)	0(0%)	3(5%)	10(17%)
Nymphaea	1(2%)	42(70%)	2(3%)	15(25%)
Crataegus	1(1%)	0(0%)	81(97%)	2(2%)

5. Discussion

The results from this work are very promising, but highlight a number of problems which must be overcome before work can proceed. The first is the difficulty caused by pollen grains lying at different levels in the gel. The depth of focus of the microscope is, of course, small compared to the pollen grains and so the image captured by the camera varies dramatically with the position of the focal plane. The use of ClearCol is beneficial but the pollen grain's z-positions are still too variable to allow large-scale testing to be carried out. A system is currently being developed which will take a number of measurements at different focal positions and produce an optimum focal position. The state of this work is such that a focus position can be achieved but the operation is time consuming, taking between 15 and 30 s per pollen grain. The majority of this time is consumed by the Quickstep in moving the stage to the various focal positions.

Secondly, the prepared slide must be fairly sparse (an example image showing typical pollen density is shown in Fig. 6). If pollen grains are too close to each other they

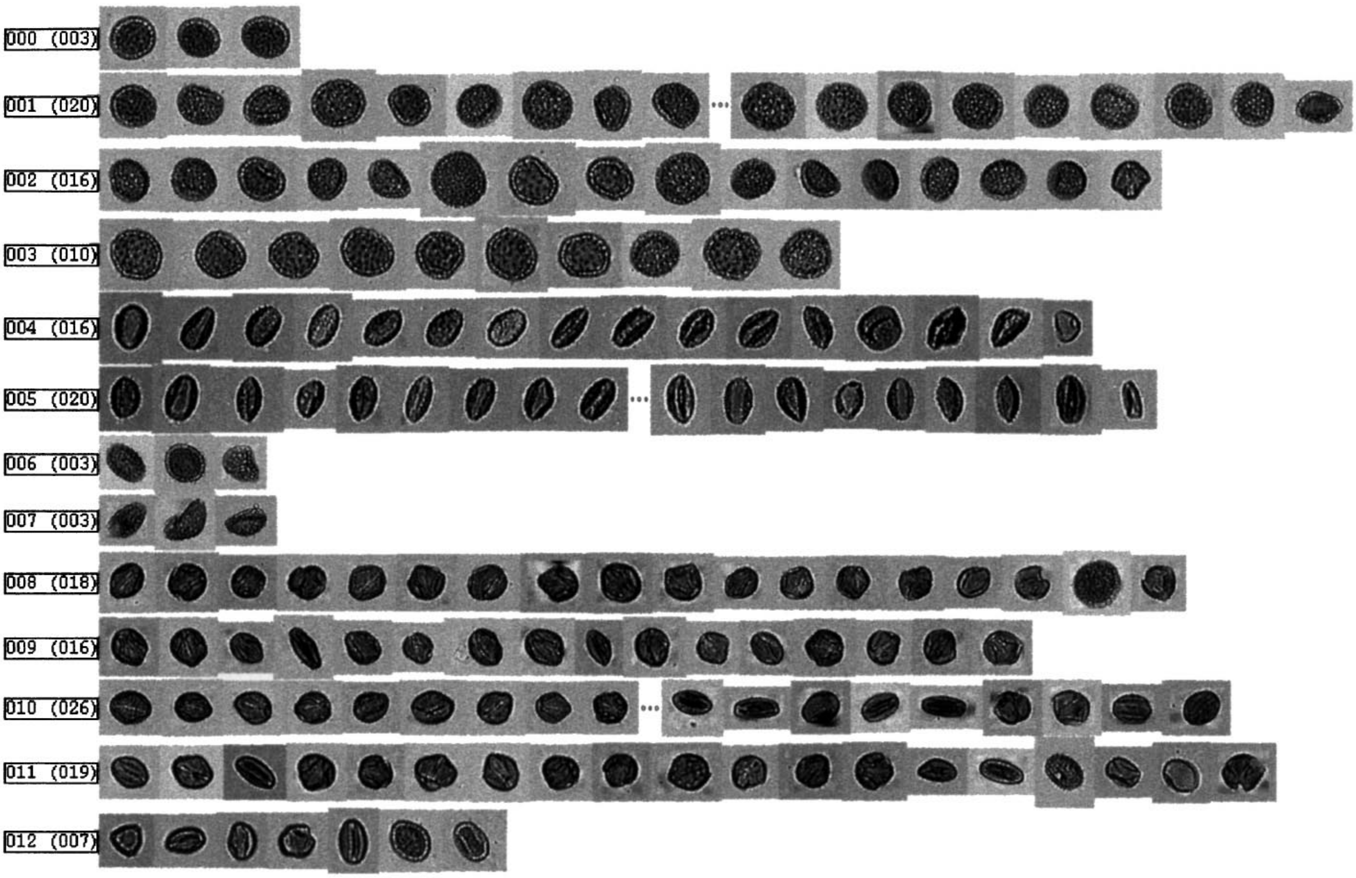

Fig. 5. The network classes generated at stage 4 of the processing, and examples of the images that were assigned to each class.

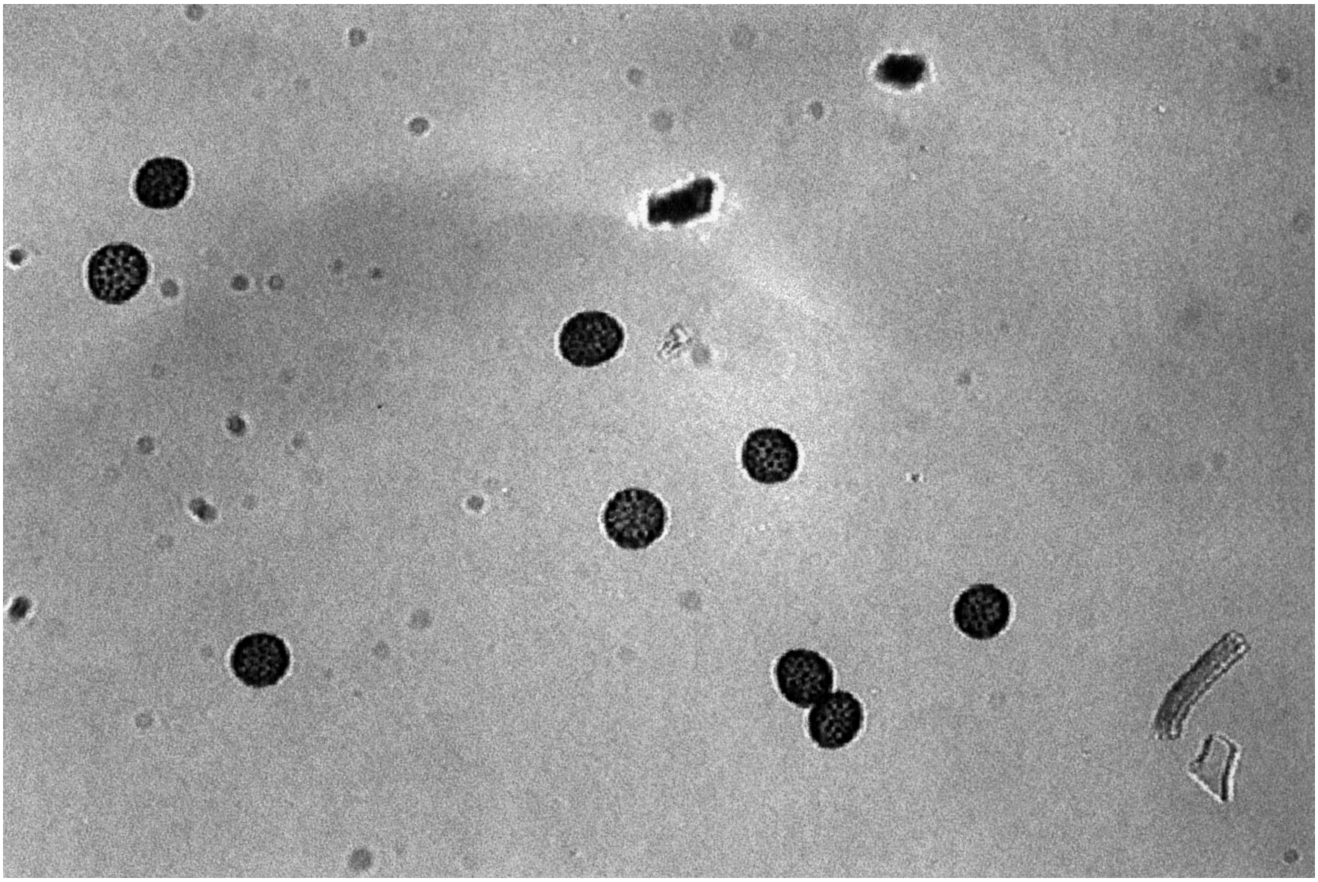

Fig. 6. An image taken at 100 × magnification showing typical pollen density.

cannot be separated by the early stages of processing as they are currently implemented. This is important as the neural network stages can only deal with a single pollen grain at a time.

Thirdly, the evaluation of any recognition system such as that proposed in this paper is problematical unless it uses a known set of test data. Although databases of pollen images do exist, and some are now even available via the world wide web, there are none which are suitable for setting up or testing the type of system described here. We have therefore used a range of reference pollens, and obtained our own images. However, if this system, or others like it are to be developed, then it would be advantageous to have standard datasets of pollen images. This would make it possible to objectively make comparisons between systems and to evaluate their efficiency. A prototype database containing the datasets used in this work is available via the WWW (this can be accessed from http://www.sees.bangor.ac.uk/research/nvrl/nvrl.html).

6. Conclusions

This paper has described a new approach to the automated identification and classification of pollen. Most previous approaches have concentrated on the problem of classifying pollen images but have ignored the problem of how to obtain suitable images in the first place. The methods used for the classification have also been inherently difficult to generalise and whilst they may give impressive results on a particular dataset will require a considerable amount of effort to achieve similar results on a different dataset. The approach presented in this paper is intended to tackle the whole problem of automating pollen counting, from obtaining images from slides to the final pollen classification. The methods employed are inherently flexible and can be expanded to include additional taxa as required.

The computationally intensive nature of image processing is a serious barrier to the automation process. In our work we have attempted to alleviate this problem by using a hierarchical approach. This leads to a system which successively removes the unwanted objects from the input dataset. The use of low-resolution images at the start of the process reduces the amount of time spent analysing detritus. High-resolution images are only used for the final classification of the pollen into classes.

For the first time a system has been presented which has the potential to take a microscope slide containing pollen grains and produce a classification of them with the need for minimal human intervention.

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