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**Automation of Pollen Analysis using a Computer
Microscope**

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Craig Alexander Holdaway

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Abstract

The classification and counting of pollen is an important tool in the understanding of processes in agriculture, forestry, medicine and ecology. Current pollen analysis methods are manual, require expert operators, and are time consuming. Significant research has been carried out into the automation of pollen analysis, however that work has mostly been limited to the classification of pollen. This thesis considers the problem of automating the classification and counting of pollen from the image capture stage.

Current pollen analysis methods use expensive and bulky conventional optical microscopes. Using a solid-state image sensor instead of the human eye removes many of the constraints on the design of an optical microscope. Initially the goal was to develop a single lens microscope for imaging pollen. In-depth investigation and experimentation has shown that this is not possible. Instead a computer microscope has been developed which uses only a standard microscope objective and an image sensor to image pollen. The prototype computer microscope produces images of comparable quality to an expensive compound microscope at a tenth of the cost.

A segmentation system has been developed for transforming images of a pollen slide, which contain both pollen and detritus, into images of individual pollen suitable for classification. The segmentation system uses adaptive thresholds and edge detection to isolate the pollen in the images.

The automated pollen analysis system illustrated in this thesis has been used to capture and analyse four pollen taxa with a 96% success rate in identification. Since the image capture and segmentation stages described here do not affect the classification stage it is anticipated that the system is capable of classifying 16 pollen taxa, as demonstrated in earlier research.

Preface

This research began as an investigation into a number of related fields, gradually gathering structure as knowledge of theory and practicalities of the various fields, particularly optics, was gathered. The aim of the project is to automate the classification and counting of pollen. This thesis is one iteration of the product development cycle. The intention in a single iteration is that it may deliver the intended results, but at worst it will make significant progress towards achieving the aim. I believe that this research has resulted in a useful prototype system for capturing pollen images, and a full system that will serve as a valuable basis for future development.

This research encompasses a range of fields including optics, microscopy, image processing, and programming. As a result it is not expected that the reader will be familiar with all of these fields to a great depth. Therefore the background section of this thesis introduces relevant information in each of the fields. That said, some fundamental background knowledge in the following areas is expected:

- High school level optics, although a little revision is given in the background.
- A general understanding of image processing, as only the specific techniques used in the segmentation are presented in the background.
- Basic programming, ideally with knowledge of C++ and MATLAB™.
- High-school level statistics, mostly for interpretation of the texture formulae, and the mean and variance filter.

A paper detailing the results of the research into capture (sections 3 and 4) was submitted and consequently presented as a poster presentation at the 2003 Image and Vision Computing New Zealand Conference held at Massey University, Palmerston North, in November [1]. This was very well received, and indeed several notable people remarked they while this idea had occurred to them, they were not sure of its virtue or correctness, but are now convinced, and may consider

[1] C. A. Holdaway, R. M. Hodgson, "Reinventing the Microscope in the Age of Digital Imaging" in *Image and Vision Computing New Zealand*, 2003, pp.286-290.

applying the idea at their establishments. Additionally a seminar was given to the IIST staff in August detailing the progress made on the image capture research, and a short summary of my research into Wavefront Coding was distributed to the IIST staff. The latter is attached at the appendix of this thesis.

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