

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

An Automated Pollen Recognition System

A Thesis submitted to
Massey University, Turitea, Palmerston North, New Zealand
in fulfilment of the requirements for the degree of
Master of Engineering.

By
Gary Allen

December 2006

Institute of Information Sciences and Technology



Massey University

Abstract

A system was developed with the aim of demonstrating that the tedious tasks of classifying and counting pollen on slides could be performed automatically to a standard comparable with that of human experts. Automation of pollen classification and counting will advance the science and range of applications of palynology.

The system developed is a completely functioning prototype. After initial set up and training it is automatic in operation.

System tests have demonstrated that the concept is viable and that the prototype developed is at a stage that it is of practical use to palynologists. There are opportunities for improvements and added functionality. Now that the system is developed and characterised, it provides a benchmark for gauging the efficacy of future improvements and adaptations.

The system is presently adaptable to many different classification problems within palynology and would be adaptable for other automated microscopic classification or imaging tasks.

Acknowledgements

Thesis supervisors were Prof. Bob Hodgson and Rory Flemmer and their efforts are well appreciated.

Special thanks go to Prof. Bob Hodgson, who offered the project to me, and spent many hours in helpful discussions and reading reports and drafts.

The Pollen Research Team at Massey University includes: Prof. Bob Hodgson, Prof. John Flenley, Prof. David Fountain, Dr. Stephen Marsland, Greg Arnold. They are an integral part of this project and provided sound and experienced advice and help along the way.

Thank you to Dr. Roger Brown who checked my work on depth of field.

Many thanks are owed to Steve Denby and the crew at the special works and mechanical workshop of the Institute of Fundamental Sciences, Massey University, who built the AutoStage.

Manual pollen counters were: Xun Li (PhD), Kevin Butler (laboratory technician), Alistair Clements (student), Prof. John Flenley and Prof. David Fountain. Many thanks for the time taken to count these slides and again to David, for supplying the pollen for the slides.

Thanks to Xiuying Zou for conventional image capture of reference pollen.

And to my wife, Linda, who encouraged and supported my decision to study again, my love.

Contents

ABSTRACT	I
ACKNOWLEDGEMENTS	II
CONTENTS	III
LIST OF TABLES AND FIGURES	V
1 INTRODUCTION	1
1.1 AIMS AND OBJECTIVES	3
1.2 DESIGN SPECIFICATION: BASIC REQUIREMENTS	3
1.3 CONTRIBUTION OF THE AUTHOR TO THIS PROJECT	5
1.4 PUBLISHED PAPER	6
2 BACKGROUND.....	7
2.1 PALYNOLOGY – THE STUDY OF POLLEN	7
2.2 MICROSCOPY	10
2.3 AUTOMATED POLLEN RECOGNITION.....	12
2.4 NEURAL NETWORKS	13
2.5 SYSTEM BUILDING	17
3 THE CAPTURE OF AN IMAGE FROM A SLIDE	19
3.1 OVERVIEW	19
3.2 THE SLIDE.....	20
3.3 THE XY STAGE: SPECIFICATION AND OPERATION.....	21
3.4 AUTO-FOCUS	24
4 MICROSCOPES.....	31
4.1 LOW MAGNIFICATION DIGITAL MICROSCOPE	31
4.2 HIGH MAGNIFICATION DIGITAL MICROSCOPE	33
5 LIGHTING	44
5.1 DARK FIELD ILLUMINATION.....	45
6 THE SEGMENTATION OF POLLEN.....	48
6.1 SEGMENTATION AND SEGMENTATION OF TOUCHING OBJECTS	48
6.2 THE SEGMENTATION OF LOW MAGNIFICATION IMAGES	49
6.3 THE SEGMENTATION OF HIGH MAGNIFICATION IMAGES.....	52
7 FEATURES EXTRACTION AND CLASSIFICATION OF POLLEN	55
7.1 POLLEN FEATURES	55
7.2 CLASSIFICATION USING AN ARTIFICIAL NEURAL NETWORK	56
7.3 AUTO STAGE REPORTS	60
8 TESTING AND COMPARISON WITH EXPERTS	61
8.1 CLASSIFICATION TESTS	62
8.2 A COMPLETE SYSTEM TEST	69
9 THESIS CONCLUSIONS AND FUTURE WORK	77
9.1 THESIS CONCLUSIONS	77
9.2 FINAL STATE OF THE PROJECT	77
9.3 FUTURE WORK.....	78
10 BIBLIOGRAPHY	84

11 APPENDICES	11-1
A. DEFINING DEPTH OF FIELD FOR AUTOStAGE	11-2
B. PUBLISHED PAPER	11-5
C. DATA SHEETS	11-12
D. RAW DATA FROM VERIFICATION TESTING	11-14
E. MLPFWD – NETLAB HELP FILE	11-15
F. MLP - NETLAB HELP FILE.....	11-16
G. SOFTWARE DESCRIPTION	11-18
H. SOFTWARE SOURCE CODE – MATLAB M FILES	11-23

List of Tables and Figures

TABLE 4-1: MICROSCOPE OPTICAL DATA WITH SYMBOLS USED IN FORMULAE BELOW.....	33
TABLE 8-1: IMAGES OF POLLEN TYPES USED IN VERIFICATION TESTING	70
FIGURE 1-1: THE AUTOSTAGE PROTOTYPE	2
FIGURE 2-1: A SECTION OF POLLEN WALL SHOWING STRUCTURE AND SOME FEATURES.....	8
FIGURE 2-2: GOLDEN ROD (ECHINATE), OAK POLLEN(COLPI) AND BIRCH POLLEN (PORES). SCANNING ELECTRON MICROSCOPE (SEM) IMAGES OF POLLEN FROM “NATIONAL POLLEN AND AEROBIOLOGY RESEARCH UNIT” WEB SITE[58].	9
FIGURE 2-3: POLLEN GRAIN ON A CAMELLIA PETAL AT 1500X FROM A COMMERCIAL (KEYENCE) DIGITAL MICROSCOPE	11
FIGURE 2-4: OLYMPUS BX51 AS USED TO CAPTURE CONVENTIONAL MICROSCOPE IMAGES FOR COMPARISON STUDIES	11
FIGURE 2-5: A MODEL OF A NEURON [30].....	14
FIGURE 2-6: THREE SIMPLE ACTIVATION FUNCTIONS, ϕ : FROM LEFT TO RIGHT: A) THRESHOLD, B) PIECEWISE-LINEAR, C) SIGMOID.	14
FIGURE 2-7: FULLY CONNECTED FEED-FORWARD NETWORK WITH ONE HIDDEN LAYER [30].....	15
FIGURE 2-8: DATA FOR SIZE VERSUS ROUNDNESS OF TWO POLLEN TYPES GRAPHED	16
FIGURE 3-1: ELEMENTS OF AUTOSTAGE IMAGE CAPTURE.....	19
FIGURE 3-2: GLASS SLIDE SHOWING REFRACTION EFFECTS.....	20
FIGURE 3-3: DIAGRAM OF LOW MAGNIFICATION IMAGES ON A SLIDE.....	23
FIGURE 3-4: LOCATION OF AN OBJECT IN AN IMAGE FROM THE SLIDE REFERENCE POINT. R AND C ARE IN PIXELS. PIXEL DISTANCE FROM THE CENTRE IS CALCULATED AND CONVERTED TO STEPS TO BE ADDED TO X AND Y, KNOWN IN STEPS.	24
FIGURE 3-5: A GRADIENT SQUARED MEASURE OF FOCUS SHOWING THE THREE FOCUS LEVELS OF A SLIDE. THE PEAKS FROM LEFT TO RIGHT INDICATE: THE SLIDE BOTTOM, THE SLIDE/COVER-SLIP WITH THE POLLEN SUSPENSION, AND THE TOP OF THE COVER SLIP. THE SLIDE IS QUITE DIRTY WHICH IS INDICATED BY THE OUTER SURFACES HAVING MORE DETAIL AND THEREFORE HIGHER FOCUS MEASUREMENT VALUES.	25
FIGURE 3-6: FOCUS FUNCTION. THIS FIGURE FROM GROEN ET AL. [28], DEFINES THE PROPERTIES REQUIRED OF A GOOD FOCUS FUNCTION. v SHOULD BE SMALL AND REPRODUCIBLE WHILE η IS LARGE, IDEALLY $\epsilon = 0$. THIS FIGURE CAN BE USED TO EVALUATE THE GRAPHS IN FIGURE 3-10.	26
FIGURE 3-7: FOCUS MEASURES; SQUARED GRADIENT COMPARED TO VOLLATH5 [39].....	27
FIGURE 3-8: CROPPED IMAGE SERIES; #18, #19, #20. #19 WAS SELECTED AS ‘IN-FOCUS’ BY THE FOCUS ALGORITHM.	28
FIGURE 3-9: THE ENTIRE IN-FOCUS IMAGE, #19 OF SERIES USED TO PRODUCE ASSOCIATED GRAPHS, THE FIRST OF WHICH IS SHOWN ABOVE, TO THE RIGHT . MORE GRAPHS OF FUNCTION VALUES VERSUS FOCUS STEP ARE SHOWN IN FIGURE 3-10, AND A SECOND IMAGE SERIES EXAMPLE IS SHOWN IN FIGURE 3-11.	28
FIGURE 3-10: FOCUS GRAPHS; FOCUS ALGORITHM VALUES VERSUS IMAGE SERIES NUMBER. THE FOCUSED IMAGE IS SHOWN ABOVE. A CROSS MARKS ANY LOCAL PEAKS AND A DOTTED LINE ACROSS MARKS A NOISE FLOOR CALCULATED LEVEL, BELOW WHICH PEAKS ARE IGNORED. ALL ALGORITHMS DETECT IMAGE #19 BUT THE SHAPES VARY AS SHOWN.	29
FIGURE 3-11: FOCUS GRAPHS FOR A SECOND IMAGE (SHOWN BELOW)	29
FIGURE 3-12: A SECOND IMAGE SERIES USED FOR FOCUS DATA: IN-FOCUS IMAGE #21.....	30
FIGURE 4-1: LOW MAGNIFICATION MICROSCOPE IMAGES SHOWING, FROM LEFT TO RIGHT, 100MICRON SPACED LINES AND POLLEN ON A SLIDE. THE WHITE MASS NEAR THE EDGE IS A WAX SEAL.	32
FIGURE 4-2: SCANNING ELECTRON MICROSCOPE (SEM) IMAGES OF SCOTS PINE GRAIN (LEFT) BIRCH POLLEN GRAIN (CENTRE) GRASS POLLEN GRAIN (RIGHT). SCALE IS UNKNOWN. [58].....	35
FIGURE 4-3: AN INTERESTING COMPARISON OF POLLEN CAPTURED ON AUTOSTAGE, COMPARED TO THE SEM IMAGES IN FIGURE 4-2. FROM LEFT TO RIGHT THEY ARE PINE (RADIATA), SILVER BIRCH, AND GRASS (BROWN TOP) A SILVER BIRCH POLLEN MAY VARY FROM 15 TO 28 MICRONS DIAMETER. THE PINE POLLEN ARE ABOUT 50-70 MICRONS ACROSS. RELATIVE SCALE OF IMAGES IS APPROXIMATE ONLY.....	35

FIGURE 4-4: FIELD CURVATURE DISTORTION	36
FIGURE 4-5: CIRCLE OF CONFUSION FOR DEPTH OF FOCUS	36
FIGURE 4-6: LIGHT THROUGH AN APERTURE SHOWING AIRY DISK, DIFFRACTION PATTERNS.	37
FIGURE 4-7: AIRY DISK INTENSITY PROFILES (INTENSITY VERSUS DISTANCE ACROSS DISK) SHOWING SPARROW CRITERION	38
FIGURE 4-8: GLASS SLIDE SHOWING REFRACTION EFFECTS.....	39
FIGURE 4-9: RAY DIAGRAM FOR DEPTH OF FIELD. AN IMAGE FORMING LENS AND ITS HALF ANGULAR DIAMETER, β . IMAGE DISTANCE, v , DIVIDED BY OBJECT DISTANCE, u , IS MAGNIFICATION, M . FOR A GIVEN CIRCLE OF CONFUSION ABOUT THE IMAGE POINT, THERE IS AN ASSOCIATED DEPTH OF FIELD AROUND THE OBJECT POINT. A THICK LENS SIMPLY CREATES TWO PRINCIPAL PLANES THAT AFFECT THE VALUES OF PARAMETERS BUT DO NOT AFFECT THE RELATIVE MEASURES USED IN THE ASSOCIATED EQUATIONS.	41
FIGURE 4-10: HIGH MAGNIFICATION MICROSCOPE CONSTRUCTION.....	43
FIGURE 4-11: HIGH MAGNIFICATION IMAGES CROPPED TO THE CENTRAL PORTION (500x500) OF THE IMAGE. THE POLLEN ON THE LEFT IS ABOUT 40 MICRONS ACROSS AND THE PAIR IN THE RIGHT HAND IMAGE ARE ABOUT 20 MICRONS DIAMETER.	43
FIGURE 5-1: LIGHT-FIELD AND DARK-FIELD ILLUMINATION (RESPECTIVELY) SHOWING POLLEN GRAINS STANDING OUT MORE AMONGST DETRITUS IN THE DARK-FIELD IMAGE.....	46
FIGURE 5-2: DARK-FIELD LIGHTING. LEFT: IMPLEMENTED. RIGHT: SIMPLE ALTERNATIVE - IN THREE DIMENSIONS THIS LIGHTING FORMS A HOLLOW CONE OF LIGHT WITH APEX AT THE OBJECT AND THE OBJECTIVE LENS INSIDE THE HOLLOW.....	46
FIGURE 6-1: EXAMPLES OF CLUMPING. THE IMAGE ON THE LEFT SHOWS CLUMPING WITH OVERLAPPING AND ON THE RIGHT, POLLEN GRAINS OVERLAP WITH DETRITUS. THE TRANSLUCENT NATURE OF THE POLLEN IS APPARENT IN THESE IMAGES.	49
FIGURE 6-2: TWO LOW MAGNIFICATION IMAGES AND THEIR SEGMENTED IMAGE BELOW, FROM A SERIES OF IMAGES OF A SLIDE OF FOSSIL POLLEN FROM A CORE SAMPLE TAKEN FROM EASTER ISLAND BY PROF. JOHN FLENLEY.	50
FIGURE 6-3: IMAGES OF SEGMENTATION SEQUENCE. TOP LEFT TO BOTTOM RIGHT ARE:	51
FIGURE 6-4: ILLUSTRATION OF THE SAME POLLEN BEING SELECTED TWICE. THE LEFT IMAGE IS EVALUATED FIRST, THEN THE RIGHT IMAGE. THE LARGER POLLEN IS THE TARGET IN THE FIRST IMAGE AND THE SMALLER POLLEN IS THE TARGET IN THE SECOND. THE TARGET POLLEN GRAINS ARE TENDING TO APPEAR BELOW AND LEFT OF CENTRE OF THE IMAGE. IF THE CENTRE, OR POSITION AT WHICH A POLLEN IS EXPECTED, IS ALTERED TO BE BETWEEN THE LAST FOUND POLLEN AND TRUE CENTRE (SHOWN AT THE NARROW CROSS) THEN THE CORRECT POLLEN IS MORE LIKELY FOUND EACH TIME.....	53
FIGURE 7-1: UNDER/OVER-FITTING: THE EXAMPLE DATA POPULATION IS SINUSOIDAL. THE DATA CAN BE FITTED WITH A STRAIGHT LINE, A SINUSOID, OR A POLYNOMIAL OF SUFFICIENT ORDER TO CUT EVERY POINT EXACTLY. THE POLYNOMIAL MODELS ANY NOISE PRESENT AND IS THUS "OVER-FITTED", AS IT DOES NOT REPRESENT THE POPULATION DATA AS WELL AS THE SINUSOID.....	57
FIGURE 7-2: OPTIMISATION BETWEEN EARLY-STOPPING AND OVER-FITTING.....	59
FIGURE 8-1: SLIDE-A RESULTS.....	71
FIGURE 8-2: SLIDE-B RESULTS	71
FIGURE 8-3: SLIDE-C RESULTS.....	72
FIGURE 8-4: SLIDE-D RESULTS.....	72
FIGURE 8-5: DATA POINTS FOR ALL TESTS. SLIDES A, B, C, D ARE IN COLUMNS; POLLEN TYPES PNN ARE IN ROWS; X-AXIS IS POLLEN COUNT; Y-AXIS IS PERSON/MACHINE.	73
FIGURE 8-6: OLYMPUS BX61. A COMMERCIAL AUTOMATIC MICROSCOPE WITH DIGITAL CAMERA, AUTO-FOCUS, XY STAGE MOVEMENT AND SLIDE STACKER VALUED AT ABOUT \$AUS150,000	75
FIGURE 9-1: THE TWO MOST IN-FOCUSSED IMAGES OUT OF NINE USED FOR FOCUS INTEGRATION (LEFT AND CENTRE) AND THE RESULT (RIGHT)	79
FIGURE 9-2: DARK FIELD ILLUMINATION USING DETUNED INTERFERENCE FILTERS.....	83
FIGURE 11-1: CIRCLE OF CONFUSION AND DEPTH OF FIELD	11-2
FIGURE 11-2: THE AIRY DISK AND ITS LIMITS OF MOVEMENT ON THE IMAGE SENSOR THAT WILL DEFINE A CIRCLE OF CONFUSION USED FOR DEPTH OF FIELD CALCULATION.	11-3
FIGURE 11-3: IMAGE SIDE RAY DIAGRAM SHOWING DEPTH OF FOCUS AND CIRCLE OF CONFUSION	11-4