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Imaging of Potato Shoots for Automated Mass Micropropagation

K. Peleg, M. Ziv, J. Sklansky, F.J. Hoffmann

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Title

IMAGING OF POTATO SHOOTS FOR AUTOMATED MASS MICROPROPAGATION

Investigators' Names

(Principal listed first)

KALMAN PELEG

MEIRA ZIV

JACK SKLANSKY

FRANZ HOFFMANN

VICTOR ALCHANATIS

ODED COHEN

MARK VRISENGA

Investigators' Institutions

TECHNION

UNIV. OF JERUSALEM (RHOVOT)

UNIV. OF CA IRVINE

UNIV. OF CA IRVINE

TECHNION

TECHNION

UNIV. OF CA IRVINE

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Signature

Principal Investigator

Signature

Institution's Authorizing Official

FELIX NAGGAR

MANAGER PROJECTS PROMOTION
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ABSTRACT

Tissue cultures find increasingly widespread applications for cloning of many plants. Commercial propagation by tissue cultures is limited to ornamental plants because the cost of the required skilled labor can not compete with conventional propagation methods. To cut down the cost, some automation is essential. To this end, a cup conveyor, machine vision based, automatic subculturing system was proposed, and simulatively applied for potato tissue cultures. This is a simpler and more cost effective solution, than the popular trend of imitating manual subculturing tasks by robots.

Automation in plant micropropagation can be greatly simplified if the propagated plantlets have some morphological properties that facilitate automatic chopping and subsequent inspection and classification of the pre-cut plantlet segments by machine vision as viable propagules. We were able to control the morphogenic pattern of in vitro propagated potato plantlets by adding various concentrations of ancymidol to the nutrient solution. It was found that plantlets cultured in 0.25ppm ancymidol best fit the requirements for automated mass micropropagation.

Plantlet segment propagules were generated, by random chopping of whole potato plantlets by a simple mechanical guillotine. Initial experiments for chopping plantlets by a laser beam were also conducted. The randomly chopped plantlet segments were inspected by a machine vision system and classified as viable propagules or rejects. An algorithm was developed for determining optimal illumination conditions for the machine vision system, which enhances the color contrast between the stem and the leaves. As a preamble to automatic chopping, a task-focused modeling procedure was proposed, for finding optimal cutting locations along the stem, instead of random chopping.

Initially, plantlet segments were classified by a simple algorithm that took advantage of the inherent difference between the geometric shape and gray scale levels of the stems and the leaves. The resulting classification error was 12%. Using color machine vision and a Neural Network based classifier, the classification error was decreased to 7%. By combining color with geometric features the classification error was further decreased to 5%. Finally, a classifier voting algorithm was developed and applied, that yielded a classification error of just 1.4%. This is exceptionally good, given the very high variability of the plantlet segments.

In summary, we have laid a basis for a practical system, which may be used for implementing a prototype of an automatic subculturing system of potato plantlets from tissue cultures.

Chapter 1 DESCRIPTION OF PROBLEM

Tissue culture techniques are finding increasingly widespread applications for clonal propagation of many plants. Protocols for mass propagation of genetically identical, disease and virus free plants, have been developed for many species. Conger (1986), compiled detailed tissue culture propagation procedures for ornamental and tree species as well as fruit, vegetable and several agronomic crops. Plant propagation by tissue culture, usually involves three main stages: Stage I: "Aseptic explant establishment in vitro" - a suitable explant, depending on the species involved is prepared; following disinfection, the explant is planted in a suitable nutrient medium, which enhances growths and the initiation of buds and/or shoots. Stage II: "Rapid multiplication" - material obtained in stage I is transferred into a nutrient medium containing appropriate proportions of phytohormones for enhancing rapid development of shoots, e.g. either axillary, and/or adventitious shoot buds, Murashige (1978), Ziv (1983). The procedure can be continued indefinitely. In many species, plant organs, e.g. roots, shoots, tubers or bulbs may be induced to form on tissues which normally do not produce these organs. This form of adventitious organogenesis has a higher potential than the induction of axillary buds for mass clonal propagation of plants. Stage III: "Hardening" - the plantlets or organs obtained in stage II may be either subcultured again or transferred to a hardening medium, before transfer to the soil, (Murashige 1974, 1978), Ziv (1986). In some cases, shoots induced in culture may spontaneously produce roots, whence they may be directly transferred to the soil. An alternative approach to organogenesis is the pathway of embryogenesis, from isolated explants or callus tissue, which can produce somatic embryos in vitro, Murashige (1978), Ziv (1986). Somatic embryos can be used as artificial seeds as reported by Walker (1986).

In spite of its advantages, large scale commercial plant propagation by tissue cultures is largely limited to ornamental plants, even though culture protocols for many additional economically valuable plant groups are readily available. This is mainly due to the intensive skilled labor required for subculturing the stage II propagules and in transferring individual shoots or plantlets into and out of stage III culture containers. Anderson et. al. (1977) estimated that labor accounts for 60%-65% of tissue culture costs. The production costs, therefore, can not compete with the conventional propagation methods, in the majority of vegetable, agronomical and fruit crops. In order to cut down the production costs, the development of a certain degree of automation in stage II and III is essential, as outlined in the objectives of the present proposal. Potato is a vegetatively propagated crop plant, which is perpetuated mainly through tuber production. The continued use of vegetative material requires stock renewal while eliminating pathogens, Cassells and Long (1982). Currently tissue culture methods have been developed for potato propagation either through plantlet or micro tuber production, Miller and Lipschutz (1984), Roca, Bryan and Roca (1979). The system is presently not economical because of intensive hand manipulation (as mentioned), and the low rate (only one or two per shoot) of microtuber production, Hussey and Stacey (1984), Thomas (1981). In addition, the long agronomical handling (seedling in greenhouses, bedding and field transplanting) before transplanted plants are established, also contribute to the high cost of the finished product. One way to obviate these problems is reducing manual handling and increase

tuber production by image recognition of a potential shoot and culture manipulation to increase micro tuber yield.

Two main approaches may be followed in automating stages II and III of tissue culture processes:

- a. Robotic manipulation of each and every plantlet and shoot piece from tissue culture, effectively trying to duplicate existing manual subculturing operations.
- b. Develop new plantlet morphology, more suitable for conventional automation, by en masse handling, on a conveying production line system.

The first approach was already tried by some companies, as described by Bry (1986) Massen et.al. (1988), Deleplanque et.al. (1985), Kutz (1986), Watake and Kinase (1988), Miwa et.al. (1988), Simonton (1991), Kinase and Watake (1991) and criticized by Slius (1985). The second approach is advocated herein, because of the following reasons:

1. Manipulation speed of current robots can not be much faster than manual subculturing, while their versatility and dexterity is inferior by far to human operators.
2. Suppose optimistically, that the robot works two shifts per day, at the peak manual limit, of say 5,000 operations or transfers per shift, (Walker 1986), i.e. one subculture every 5.76 seconds. A conservative estimate of the investment in such a robot is about \$100,000. Assuming manual subculturing labor costs of \$20,000 per year, (two shifts), the cost recovery of the robot would be 5 years at best, while its technological obsolescence will probably be about 2 years. Note that the above figures do not include interest, maintenance and skilled labor costs for operating and supervising the proper functioning of the robot. Furthermore, the robot will be able to perform only part of the subculturing tasks, hence some part of the process will still have to be done manually, at additional cost.
3. If the morphology of the plantlet and/or storage organ, can be changed by biological means, to be more suitable for semi-guided or even random mechanical chopping, then the cut pieces could be transported on a suitable conveyor and inspected by computer vision for presence or absence of propagation organs, e.g. buds and shoots, as described in Peleg (1987, 1988). Pieces without propagation organs would be rejected, while those with buds or shoots could be automatically deposited into appropriate nutrient media, in vials or other culture containers. The whole process can be accomplished in aseptic conditions, including automatic supply and placement of culture vessels on the conveyor, as well as opening and closing lids, by well established existing technology in the food industry.
4. The economics of such a system are much more favorable, as per the following example. Existing commercial, computer vision hardware could easily examine 8 pre-cut tissue culture pieces per second, moving on a small two-lane conveyor, at a moderate linear velocity corresponding to say four pieces per second. If only 50% of the pre-cut pieces will incorporate suitable propagation organs, then the net throughput will be about 4 subcultures per second, i.e. $4 \times 3600 \times 8 = 115200$ per shift, which is 23 times faster than the above estimated 5,000 pieces per shift of the robot. Yet the

cost of such a machine will probably not be much more than the cost of a robot.

Figure 1B depicts conceptually, an automatic machine for subculturing tissue culture plantlets. Briefly, the operation of this machine may be described in three stages, as follows:

I. Initially, bunches of plantlets will be manually removed from growth containers and chopped into approximately 1 cm. long segments. We have successfully tried this operation by using a simple, office paper cutting guillotine. The blades of the guillotine can be periodically flame sterilized, similarly to the scalpels in manual subculturing. Eventually, this operation will also be automated and made more efficient, by automatic chopping. Images of plantlets traveling on a conveyor belt will be analyzed by machine vision and optimal cutting sites will be selected, such that the maximal number of propagules is obtained from each plantlet.

A laser beam will be directed to the selected cutting sites, whence clean and sterile cuts can be effected, Holdgate (1992). Note that in the meantime, the chopping operation is not shown in fig. 1B.

II. Regardless of the chopping mode, the in-feed to the machine is a stream of pre-cut plantlet segments (1) which are deposited into a trough (2). A singulating conveyor (3) will pick up plantlet segments from the trough, preferably one segment at a time, or bunches of say two, three or four segments, which will be deposited into a cup conveyor (5). The singulation process will be further facilitated by an air stream (4), which will assure that the number of picked up plantlet segments, that are deposited into each cup, will be between one and three, in most cases.

III. A video camera (6), will transfer to a computer, the images of the plantlet segments traveling in the conveyor cups. The images will be analyzed and classified by the computer, according to the number of plantlet segments in the cup and the presence or absence of propagation organs on each segment, if any. Accordingly, the computer will issue a signal, to trip the cup (7), over the appropriate cross conveyor (8), whence the plantlet segments will be dropped into nutrient filled growth containers (9). The containers will then be automatically closed by air tight covers and transported to the growth room, whence a new crop of plantlets will grow.

In fig. 1B a two lane machine is depicted. The machine vision system will analyze the contents of two cups at a time, whence a throughput of 10 cups per second could be easily realized. Most of the cups will contain 1 to 3 plantlet segments, some may contain more than 3 plantlet segments, while others may be empty. Below the cup conveyor (5), a four lane cross conveyor (8) is depicted, whence four nutrient filled growth containers (9), per each cup conveyor lane are available for depositing the contents of each cup. For best results about 10 plantlet segments will be dropped into each growth container. Thus, the ultimate classification task will include an optimal dropping schedule of the cup contents, such that each container is filled by 10 viable plantlets approximately, before it is sent to the closure operation.

There are three possible cup contents: a. All plantlet segments are good propagules, whence they will be dropped into one of the nutrient filled containers (9). b. The cup may contain a mixture of good and bad plantlet segments, whence they will be dropped onto conveyor (10) and recycled back to the in-feed conveyor (1). c. All the plantlet segments in the cup are bad propagules, in which case they will be rejected by tripping the cup over the last cross conveyor (11).

It should be noted that most of the technology for manufacturing the components of this machine is commercially available, excluding some parts of the plantlet segment singulator section (3) and the machine vision algorithms. Cup conveyor systems as in fig. 1B are routinely used in automatic fruit sorting machines, such as color sorting of apples by machine vision, at about 5 fruits per second per lane. Similarly, automatic filling of nutrient into growth containers and their hermetic closure, after the plantlets have been dropped therein, can be readily accomplished by equipment routinely used in the food industry, such as automatic filling of jam into jars. There is no sanitation problem, since the materials and the construction of the machine are very similar to those used by the food industry. Adequate sterilization can be obtained by installing the machine in a "Clean Room", and by enclosing it in a laminar flow hood, as is the custom in manually operated tissue culture labs.

Figure 1b. Machine vision controlled automatic subculturing system.

Chapter 2 GOAL OF PROJECT

The main goal of the project, was to provide a scientific knowledge base, for enabling development of a commercial system for automated subculturing of potato and other selected plants by tissue culture.

SPECIFIC OBJECTIVES OF THE PROJECT

BIOLOGY

Develop plantlet morphology which is particularly suitable for automatic chopping into uniformly sized plantlet pieces, and classification by computer vision on a conveying production line system.

1. Using chemical, biological and environmental treatments develop plantlets uniform in size and shape, with long and straight stems, while the distances between the leaves along the stems are uniform and relatively large.
2. Increase the color difference (hue and/or intensity) between the leaves and the stems as much as possible.
3. Develop nutrient composition and viscosity suitable for subculturing by randomly dropping of plantlet pieces into growth containers without loss of propagation efficiency.

MACHINE VISION

1. As a basis for subsequent automatic subculturing of plantlets on a conveyor belt, develop robust algorithms for classification of manually pre-cut plantlet pieces.

The feature extraction part of the algorithm should provide a count of the number of viable propagation organs on each plantlet piece and its size, as well as the number of plantlet pieces in the examined image. Using this information, the classification part of the algorithm will grade the image in light of optimal subculturing requirements, i.e. the plantlet or group of plantlets will be directed to the appropriate subculturing container, re-directed to the chopper or singulator units, or rejected as unsuitable for subculturing.

2. As a preamble to automated plantlet chopping, develop algorithms for locating the optimal cutting sights in images of single plantlets and groups of whole plantlets, as a function of the positions, numbers and distribution of propagation organs.

MECHANICAL

The original proposal to BARD included construction and testing of a single lane prototype machine for investigating the mechanical aspects of singulation, chopping and transportation of pre-cut plantlet pieces on a cup conveyor belt.

This part of the project was very costly, so due to the drastic reduction of the originally requested budget, it was impossible to achieve this goal in full. However, conceptual drawings are provided so that a potential commercial company can develop the machine, while using the scientific knowledge base generated by this project.

Chapter 3 BIOLOGY

INTRODUCTION

In order to make the automatic chopping process effective and the inspection task of the computer vision algorithms feasible, the plantlet should have some desirable morphological properties. Internode length for effective automatic chopping will have to be about 10mm. High color contrast between leaves and stems is essential for identifying plantlet segments that contain axillary buds, which are located at the nodes. The stems should be preferably straight, thick and light green, while the leaves should be relatively large and dark green.

Growth retardants, inhibitors of gibberellin biosynthesis, are known to regulate plant growth, enhance chlorophyll synthesis, reduce internode elongation and leaf expansion (Grossman 1990, Ziv & Ariel 1991). In potato, ancymidol (cyclopropyl-4-methoxy pyrimidin-5-yl-benzylalcohol, Reducymol, Lilly France) was reported to inhibit shoot growth and enhance tuber formation (Shemesh 1989, Perl et al 1991). The need to control the plant morphogenic pattern of *in vitro* propagated potato plants for automated classification by machine vision promoted the use of ancymidol for shoot growth regulation and enhanced chlorophyll synthesis.

Microtubers are the final product of the propagation process as well as a convenient form of long term storage of biological matter. Several studies have been conducted on induction of potato microtubers from shoot segments taken from plantlets grown *in vitro* (Shemesh 1989, Perl et al 1991, Harvey et al 1991). It was found that growth retardants, including ancymidol, stimulate tuberization. In this study we used ancymidol to control the plantlet morphology in the micropropagation stage, as well as to affect the subsequent formation of microtubers.

The effect of ancymidol treatments on the morphology of tissue cultured potato plantlets and of these treatments on microtuber formation in subcultured segments taken from plantlets treated with ancymidol is presented. The machine vision system developed for measuring the morphological features of potato plantlets and microtubers, evaluating the effects of the treatments on plantlet morphology and their evaluation by machine vision is described.

MATERIALS AND METHODS

Plant material

Potato plantlets were grown *in vitro* on MS medium (Murashige & Skoog 1962) with 0.5mg l^{-1} Nicotinic acid, 0.5mg l^{-1} Pyridoxine HCl, 0.1mg l^{-1} Thiamine HCl, 2mg l^{-1} Glycine, 30g l^{-1} sucrose, 0.1g l^{-1} Myoinositol, 0.1g l^{-1} Adenine sulfate and 2mg l^{-1} Kinetin. The pH of the medium was adjusted to 5.7 before the addition of 10g l^{-1} agar and autoclaving (120°C at 100kPa for 15 min). Fifty ml of the cooled medium was dispensed into clear plastic Magenta 6x6x8cm

containers. Ten nodal sections, including an axillary bud and a leaf from *in vitro* plantlets, were cultured in each container. The effect of ancymidol on the morphology of the plantlets was studied in a wide range of ancymidol concentrations 0ppm (control treatment), 0.25ppm, 0.50ppm, 0.75ppm and 1.00ppm. The control treatment and the two lower ancymidol concentration treatments (0.25ppm and 0.50ppm) included 70 plantlets (7 containers) and the two higher ancymidol concentration treatments (0.75ppm and 1.00ppm) included 30 plantlets (3 containers). The plantlets were cultured in a controlled environment cabinet at 25°C and 24-h photoperiod for 4 weeks. At the end of the growth period the plantlets were taken out of the containers and their morphology was evaluated by a mensuration system described in what follows.

Tuberization experiments were performed to test the tuberization potential of axillary buds from plantlets that were treated with ancymidol. Shoot segments which contained an axillary bud and a leaf were cultured in a microtuber inductive medium which consisted of half strength MS salts, 80gr l⁻¹ sucrose, 5mg l⁻¹ kinetin, 5mg l⁻¹ ancymidol and 10g l⁻¹ agar (Perl et al 1991). This medium was reported to synchronously produce microtubers in a single leaf axil of potato shoot segments. Ten shoot segments were cultured in each Magenta 6x6x8cm container, filled with 50ml medium, at 25°C and 24-h photoperiod, for 4 weeks. Shoot segments were obtained from plantlets grown in three different ancymidol concentration media: 0ppm (control), 0.25ppm and 0.50 ppm of ancymidol. At the end of the growth period the microtubers were taken out of the containers and evaluated as described in the next section.

Morphological mensuration

Evaluation of plantlet morphology was carried out by measuring some characteristic features. The features were chosen such as to indicate which plantlets are more suitable for automated chopping and inspection by machine vision. The ideal morphological parameters of the plantlet for this purpose are: dark green leaves, straight and light green stems, sufficient internode length (8–10mm) and maximal number of potential propagation organs. The plantlet features chosen to measure these parameters were:

- (a) Total length of the stem,
- (b) Number of axillary buds per plantlet.
- (c) Average thickness of the stem,
- (d) Average distance between axillary buds along the stem,
- (e) Contrast between the color of the leaves and the color of the stems,

Feature (e) indicates the propagation potential of the plantlet, i.e., how many new plantlets or microtubers can be produced.

Characteristic microtuber features were chosen to indicate: (i) The effect of the ancymidol treatments on microtuber formation, and the time for microtuber formation, (ii) Accurate estimation of microtuber size, weight and color.

These features may be used later for automatic inspection and classification of microtubers according to suitability for planting.

At the end of the four weeks growth period the microtubers were evaluated by measuring the chosen characteristic features:

- (a) Microtuber mean diameter
- (b) Microtuber weight (F/W)
- (c) Mean intensity of the three principal color components (red, green, blue (RGB)).

A computer vision system and especially developed software were used to measure the characteristic features of the plantlets and the microtubers as per the layout shown in figure 1. It comprises a video camera (Panasonic WV-D5000) which transmits a video signal of the plantlet image to a video tape recorder (VTR) (Panasonic AG7300), and simultaneously to a color image processing board (MATROX MVP-VME). The recorded image on video tape may be played back by the VTR and digitized by the image processing board in repeated image processing tests, during the software development stage. The digitized image is stored in memory for further processing (feature extraction). Two programs were developed: one to measure the characteristic features of potato plantlets, and another to measure characteristic features of microtubers. The plantlet evaluation procedure was as follows:

- (a) Each plantlet was taken out of the growth container and presented to the video camera, on a backlit plate located in an especially designed illumination chamber, as shown schematically in figure 1.
- (b) The image processing board was triggered to acquire an image of the plantlet and display the digitized image on a video monitor.
- (c) An operator marked all the axillary buds of the evaluated plantlet by moving a cursor over the plantlet image. The program counted the number of axillary buds, computed the mean internode length and the total length of the stem.
- (d) Next, the operator marked the width of the stem at three different locations along the stem whence the program calculated the sampled widths.
- (e) The mean intensities of the three principal color components (RGB) of leaves and stems were calculated at selected locations, as marked by the operator, e.g. square areas belonging to leaves or to the stem respectively.
- (f) Finally, the program calculated the difference between leaf and stem intensities of the three principal color components (RGB). The results were stored in a disk file for later retrieval and statistical analysis.

The effects of the treatments on the measured features and the statistical significance of the differences between the mean values of the measured features among the treatments were evaluated by analysis of variance. The analysis was carried out by the SAS commercial software package (procedure ANOVA), while all the hypothesis were tested at a significance level $\alpha=0.05$.

Figure 1: Schematic layout of the machine vision system used for morphological mensuration of potato plantlets.

RESULTS AND DISCUSSION

Plantlet morphology

Table 1 presents the mean values of the measured morphological features of potato plantlets cultured in 0, 0.25, 0.5, 0.75 and 1.0ppm of ancymidol. It can be seen that the mean values of the features vary considerably among the ancymidol treatments. Statistical analysis (F-test of analysis of variance) showed that the morphological variability of the plantlets among the treatments can be explained by the differences of ancymidol concentration levels. Fig 2a shows typical plantlets grown in 0.00ppm, 0.25ppm and 0.5ppm ancymidol concentrations. It can be seen that there are significant morphological differences between the plantlets treated with various ancymidol concentrations.

The plantlets cultured in the two highest ancymidol concentrations (0.75ppm and 1.00ppm) were very short at the end of the 4 weeks period: stem length ~20mm and internode length less than 3mm. The plantlets grown in 0.25ppm of ancymidol produced the longest stems, $L=7.72\text{cm}$, while the control treatment and 0.50ppm ancymidol treatment produced intermediate stem lengths, approximately 4.40cm (figure 3). It is possible that the shorter main stem in the control resulted from the simultaneous development of several secondary stems.

Figure 3 illustrates that internode length, D , decreases as the ancymidol concentration increases. The longest internode length was observed in the control treatment, 10.5mm, which did not differ significantly from the 0.25ppm ancymidol treatment, 9.1mm. The rest of the treatments had significantly shorter mean internode lengths.

The number of axillary buds per plantlet, N , was also affected by the concentration of ancymidol as indicated in figure 3. The concentration of 0.25ppm ancymidol induced the greatest number of axillary buds. It can be also observed that all ancymidol treatments produced significantly more axillary buds than the control treatment.

Treatments with low ancymidol concentration (0.25 and 0.50ppm) induced significantly thicker stems than the control treatment as indicated in figure 3. The thickness of the stems in the higher ancymidol concentrations was not measured because the small dimensions of the stem did not permit it.

The contrast between the color of leaves and stems was measured for the control and the two lower ancymidol concentrations only. It was found that the contrast between the color of leaves and stems was significantly enhanced by ancymidol (Table 1). In the ancymidol treated plantlets, the difference between intensity levels of stems and leaves in the red and green principal color components was significantly higher than in the control treatment, however, the difference between the two ancymidol treatments was not significant. This can be observed from figure 4, as indicated by the letters on the bars. The contrast of the blue component was not affected by

Table 1: Mean values of measured features of potato plantlets grown in different ancymidol concentrations. Values with different superscripts indicate significant differences at $\alpha=0.05$.

MEASURED FEATURE	ANCYMIDOL CONCENTRATION [ppm]				
	0.00	0.25	0.50	0.75	1.00
N _o OF PLANTLETS	70	70	70	30	30
SHOOT LENGTH [mm]	43.4 ^b	77.2 ^a	44.1 ^b	17.9 ^c	22.8 ^c
N _o OF NODES	5.1 ^d	9.4 ^a	8.5 ^b	7.0 ^c	7.6 ^c
INTERNODE LENGTH [mm]	10.5 ^a	9.1 ^a	6.2 ^b	2.7 ^c	2.9 ^c
STEM THICKNESS [mm]	0.8 ^b	1.2 ^a	1.3 ^a	---	---
COLOR CONTRAST BETWEEN LEAVES AND SHOOTS:					
RED COMPONENT	37.9 ^b	57.0 ^a	50.4 ^a	---	---
GREEN COMPONENT	45.5 ^b	71.7 ^a	68.0 ^a	---	---
BLUE COMPONENT	21.5 ^b	34.2 ^a	28.2 ^a	---	---

Figure 2: (a) Potato plantlets grown in 0, 0.25 and 0.5ppm ancymidol. (b) Potato microtubers formed from shoot segments taken from plantlets treated with 0, 0.25 and 0.5ppm ancymidol, and cultured on tuber induction for four weeks.

Figure 3: Effect of ancymidol on morphogenic features of potato plantlet : stem length (L), number of nodes (N), stem thickness (W) and internode length (D). Data represents the average of 70 replicate cultures per treatment for 0, 0.25 and 0.5ppm ancymidol concentrations and 30 replicate cultures per treatment for 0.75 and 1ppm ancymidol concentration; different letters within each feature indicate significantly different means at the 5% level.

Figure 4: Effect of ancymidol on color contrast between stems and leaves of potato plantlets. Data represents the average of 70 replicate cultures per treatment; different letters within each feature indicate significantly different means at the 5% level.

ancymidol at a statistically significant level.

Microtuber morphology

Tuberization in shoot segments isolated from plantlets treated with 0.25ppm and 0.5ppm ancymidol was 100% synchronized. Table 2 summarizes the mean values of characteristic features of the microtubers. Figure 2b shows typical microtubers formed after four weeks, from shoot segments taken from plantlets treated with 0ppm, 0.25ppm and 0.50ppm ancymidol. It can be seen that shoot segments taken from control plantlets that were not treated with ancymidol did not form microtuber. Microtubers formed on shoot segments taken from plantlets treated with ancymidol do not differ significantly. Statistical analysis showed that the concentration of ancymidol in which the source plantlets were grown did not affect significantly the morphology of the microtubers, in terms of weight, mean diameter and color, as summarized in Table 2. The mean weight of the microtubers at the end of a four weeks growth period was approximately 70 mg per tuber, which is significantly greater than reported in literature, 45mg per tuber at the end of a six weeks period (Harvey et al 1991). The mean diameter of the microtubers was 4.5mm. Approximately 3 buds on the microtuber surface proliferated.

Assuming perfect spherical shape of microtubers, their volume was computed and correlated to the measured weight. Figure 5 illustrates the relationship between the measured and the calculated values. It can be seen that the relationship is linear and the correlation coefficient is high: $r^2=0.95$. Therefore, the weight of the microtuber can be accurately predicted by measuring its diameter. This may enable automatic sorting of microtubers by machine vision, whereby too small microtubers will be rejected, while selecting only the sufficiently large ones for planting.

CONCLUSIONS

The main objective of this research project was to generate plantlets with uniform morphology, suitable for automated mass micropropagation, via classification by machine vision. This task can be greatly simplified if the propagated plantlets have some morphological properties that facilitate the automatic chopping process. Subsequent automatic inspection and classification of the pre-cut plantlet segments as viable propagules or unsuitable segments can also be achieved. Ancymidol was used to regulate plant growth and enhance chlorophyll synthesis. It was found that ancymidol enhances the color contrast between leaves and stems. Plantlets treated with ancymidol generated more axillary buds per plantlet than the control.

The results reported here indicate that plantlets cultured in 0.25ppm ancymidol provided the best plantlet morphology for automated mass micropropagation and the highest propagation potential. Additionally, it seems that ancymidol has a cumulative effect on the growth regulation system, that is expressed by a shorter period for microtuber formation.

Machine vision was used to evaluate the morphological changes which resulted from the biological

Table 2: Mean values of measured features of potato microtubers grown from shoot segments taken from plantlets that were cultured in different ancymidol concentrations. Values with different superscripts indicate significant differences at $\alpha=0.05$.

MEASURED FEATURE	ANCYMIDOL CONCENTRATION [ppm]		
	0.00 [†]	0.25	0.50
WEIGHT [mg]	---	79.3 ^a	66.2 ^a
MEAN DIAMETER [mm]	---	4.2 ^a	3.9 ^a
N ^o OF SPROUTING POINTS	---	2.9 ^a	3.0 ^a
RED COLOR COMPONENT	---	145.8 ^a	146.3 ^a
GREEN COLOR COMPONENT	---	144.0 ^a	143.5 ^a
BLUE COLOR COMPONENT	---	116.5 ^a	119.4 ^a

[†] No microtubers were formed

Figure 5: Correlation between measured microtuber weight (mg) and calculated volume (mm³), assuming perfectly spherical microtuber shape.

experiments. Geometrical features such as number of buds or internode length can be precisely measured and automatically logged. Some important features that till now could only be observed qualitatively, can also be measured, e.g. absolute color and color contrast. Additionally, physiological features like weight of microtubers can be accurately estimated from automatically measured geometrical features of the plantlet's image. Further development may permit fully automatic evaluation of morphogenic properties of plantlets including some additional important features, like total leaf area.

For commercial implementation of an automatic system for micropropagation, more work has to be done in several directions: (i) The cumulative effect of ancymidol on plantlet morphology has to be studied. So far, the results show that the morphology of the plantlets of the second generation grown in ancymidol, remains the same. (ii) In the suggested automatic system, the shoot segments are dropped into the containers, instead of being manually placed into the nutrient medium. The effect of random dropping on further growth of the plantlets has to be determined, simulating the dropping of the shoot segments into the growth containers. (iii) Sterility must be maintained throughout all the stages of the process, including the automatic chopping stage. Instead of designing an apparatus similar to those used by human workers (sterilized scalpels by immersion in alcohol and burning), the use of a laser beam is considered as an alternative cutting means. The various biological and engineering aspects of automatic chopping by a laser beam will have to be evaluated.

REFERENCES

- Anderson WC, Meager GW & Nelson AG (1977) Cost of propagating Brocoli plants through tissue cultures. Hort. Sci. 12, 543
- Bry LD (1986) Robots in plant tissue culture: an insight. Newsletter International Association for Plant Tissue Culture 49:2-22
- Conger BV (1981) Cloning agricultural plants via *in vitro* techniques. CRC Press, Boca Raton, Florida. 1-273
- Grossman K (1990) Plant growth retardants as tools in physiological research. Physiol. Plant, 78:640-648
- Harvey BMR, Crothers SH, Evans NE & Selby C (1991) The use of growth retardants to improve microtuber formation by potato (*Solanum tuberosum*). Plant Cell, Tissue and Organ culture, 27:59-64
- Kutz LJ (1986) Robotic transplanting. ASAE paper 86-1088

Miwa Y, Yamamoto T, Kushihashi Y & Kodama H (1988) Study on automation of plant tissue culture process – Development of automatic seedling transplantation system. J. Jpn. Soc. Precision Engineering (54)6:1107–1112 (Japan)

Murashige T & Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant, 15:473–497

Perl A, Aviv D, Willmitzer L & Galun E (1991) *In vitro* tuberization in transgenic potatoes harboring beta-glucuronidase linked to a patatin promoter: effects of sucrose levels and photoperiods, Plant Science 73:87–95

Row JW (1986) New techniques in plant tissue culture. In: Zimmerman RH (Ed) Tissue Culture as a Plant Production System for Horticultural Crops, (pp 35–45). Martinus Nijhoff Publishers

Shemesh D (1989) Bud proliferation and microtubers induction in potato (*Solanum tuberosum*) in tissue cultures. MSc thesis, Department of Agricultural Botany, The Hebrew University of Jerusalem (Hebrew)

Sluis CJ (1985) Commercialization of plant tissue culture propagation. Newsletter International Association for Plant Tissue Culture 47:2–12

Walker KA (1986) Automation of *in vitro* plant propagation: Has its time finally arrived? Genetic Engineering News, July/August:5

Watake H & Kinase A (1988) Robot for plant tissue culture. Robot 64:74–79 (Japan)

Ziv M & Ariel T (1991) Bud proliferation and plant regeneration in liquid cultured *Philodendron* treated with ancymidol and paclobutrazol. J. Plant Growth Regul., 10:53–57

Chapter 4 CLASSIFICATION OF PLANTLET SEGMENTS BY GEOMETRIC FEATURES

The machine vision classification task is to recognize and separate between good and bad propagules, as defined above. Additionally it would be advantageous to know how many leaves are connected to each stem segment. This task is complicated in three respects:

- a. There is a very large random variation of the geometry of the plantlet segments in the inspected images. Even the image of the same plantlet may vary considerably, depending on how it falls when placed in front of the camera.
- b. To be economical on a mass production scale, the ultimate sorting speed must be very fast, at least 5 double cup images per second, while using a rather inexpensive computer.
- c. The misclassification rate must be quite low, because bad plantlet segments may sometimes act as contaminants, when subcultured together with good propagules.

Two essentially different approaches may be taken for constructing a plantlet classifier:

1. The classical approach, wherein many characterizing features of good and bad plantlet segments are used as multiple dimensions feature vectors.
2. Segment the images of plantlet pieces into "leaves" and "stems" by using their difference in geometry and the rather simple connection configurations between leaves and stems.

Examples of characterizing features in images of plantlet segments that may be used by the first approach are: circumference, area, mean gray scale level, curvature, moments of inertia etc. Although these features might be quite noisy when applied to whole plantlet segments, the premise is that the distribution of the selected features is sufficiently different in the images of good and bad plantlet segments, which could enable classification with reasonable accuracy. In this approach the classifier is only as good as the selected features so one might try different features combinations to improve performance. An optimum may be found whence the dimension of the feature space does not decrease the classification speed too much, while the misclassification rate is hopefully reasonably low.

The second approach, which is the main topic of this chapter, relies on the inherent difference between the geometrical shape and gray scale level of the stems and the leaves as well as on the rather simple connectivity rules of attachment between them. This allows better classification accuracy while using but a minimal number of robust features for identifying blobs in the segmented image as leaves or stems. Whereas the first approach relies more on brute force computing power and many rather weak features, the second approach is closer to the perception of a human inspector, who would typically make a positive decision after locating at least one leaf connected to a stem. Moreover, the segmentation of the plantlet image into leaves and stems actually determines how many leaves are connected to each stem and not just whether there are any leaves connected to a stem. In the context of the present paper we concentrated on "understanding" stationary images of pre-cut plantlet segments, as a preamble for further development, of an on-line high speed plantlet sorting system.

The classical image segmentation approach begins by separating the background from the object of interest in the image by a suitable threshold gray scale level, thereby converting the image into a binary set, e.g. the background pixels are set to 0 and the pixels of the object to 1. Many algorithms have been proposed in the literature, for segmenting the resulting binary image, as for example Sklansky (1974), Rosenfeld and Kak (1982), Monga (1987), Pavlidis and Yuh-Tay (1990) and Niblack et. al. (1990). Some of these segmentation algorithms employ specialized principles, each particularly suitable for certain classes of images, while others are quite general. Of the latter, the most popular is the classical approach based on the Medial Axis Transform (MAT), e.g. Rosenfeld and Kak (1982), Niblack et.al. (1990).

Unfortunately, the MAT approach has some distinct drawbacks, which make it unsuitable for high speed processing of tissue culture images:

1. MAT representation can be redundant, i.e. some "blocks" may be contained in unions of others, and there does not seem to be any simple way of reducing this redundancy without carrying out a lengthy search process.

2. MAT representation is very sensitive to noise in the image. Even slight noise may change the "maximal-block" representations quite substantially. To overcome this problem to some extent, one has to noise-clean the image, before segmenting it.

Unfortunately this is computationally expensive, i.e. too time consuming by a low cost computer. Note also that pre-segmentation filtration may change the original plantlet image, e.g. some of the tiny connections between leaves and stems, may appear to be disconnected.

3. The very computation of MATs is very time consuming, because one needs to make many passes over the entire image. Unless one has a powerful computer incorporating array processing, this would be impractical for on line classification of tissue cultures.

We have developed a much simpler and faster image processing procedure, particularly suitable for our task, which takes advantage of the inherent difference between the geometrical shape and gray scale level of the stems and the leaves as well as on the rather simple connectivity rules of attachment between them.

The main stages of this image processing algorithm are as follows:

1. Separate the image of the plantlet from the background and find all edge pixels of the plantlet contour.

2. Compute certain characterizing features of each edge pixel.

3. Segment the plantlet image into distinct blobs by a region growing technique, using the features computed in 2.

4. Compute some primary characterizing features of each blob.
5. Pre-classify each blob as leaf or stem segment, using the primary features computed in 4.
6. From the pre-classified stem segments, if any, select one blob that is the most likely candidate for the main stem of the plantlet segment.
7. Compute some secondary features for further characterizing leaves and stems by the inherent geometrical connectivity between the blob selected in 6 and the rest of the blobs.
8. Re-classify each blob as leaf or stem, using the primary features computed in 4 and the secondary features computed in 7.

Each one of these stages is described in some detail in what follows, whence the summary section concludes the paper by evaluating the classification results on a representative set of manually pre-cut potato plantlet segments.

Features of edge pixels

We found that the segmentation task can be greatly simplified by optimal illumination, wherein for the image acquisition, the plantlet is placed on a back-lit background. By experimentally adjusting the intensity of the foreground and background illumination, it was possible to obtain sharp silhouettes of the plantlet segments while preserving the natural gray scale level difference between the stems and the leaves.

The pixels of the plantlet image and the background were easily separated by choosing a suitable threshold gray scale level. The locations of all edge pixels of the plantlet image were then found by a contour following technique, very similar to the classic chain coding algorithms for contour finding, as described in Rosenfeld and Kak (1982) and similar text books.

The premise of this algorithm is that the plantlet image consists of a group of connected pixels who's gray scale level is lower than a chosen threshold. With reference to figure 2, a first edge pixel, such as A is found by scanning the frame and comparing the gray scale level of each pixel with the chosen threshold. The steps for locating the rest of the edge pixels are:

1. Scan and record the gray scale level of a 3x3 pixel mask centered on the known edge pixel such as A, in clockwise direction.
2. The next edge pixel (clockwise), amongst the 8 pixels of the mask, such as B, is the one who's gray scale level is lower than the chosen threshold while the gray scale level of the previous pixel of the mask (anti-clockwise) is higher than the chosen threshold.

Figure 2. Image contour mapping sequence.

3. Repeat steps 1 and 2 until you return to the above first edge pixel.

Two characteristic features of each edge pixel, suffice for segmenting the plantlet image into distinct blobs. These features were termed "CURVATURE" C_{urv} and "WIDTH" W . Figure 3a is a typical analog image of a plantlet segment, while the grid in fig. 3b depicts the digitized version of fig. 3a. The tangent AB to an edge pixel i , such as P_1 in fig. 2a is defined by two neighbor pixels $(i-k)$ and $(i+k)$ along the edge of the image, such as E and F in fig. 3b. In this case $k=2$, which is a compromise between the need for noise reduction and better resolution.

The normal CD to an edge pixel i forms a cross section of the image such as P_1P_2 in fig. 3a or D_1D_2 in fig. 3b. The length of the cross sections such as D_1D_2 was defined as the width W at an edge pixel. The angle θ_p that the tangent line EF forms with the X axis is given in terms of the X,Y projections of the line EF, D_x and D_y :

$$\cos(\theta_p) = D_x/r \quad \sin(\theta_p) = D_y/r \quad r = \sqrt{D_x^2 + D_y^2} \quad (1)$$

The angle θ_n that the normal D_1D_2 forms with the X axes is expressed by:

$$\sin(\theta_n) = \sin(\theta_p + \pi/2) = \cos(\theta_p) \text{ or by } \cos(\theta_n) = \cos(\theta_p + \pi/2) = -\sin(\theta_p) \quad (2)$$

The curvature C_{urv} at a pixel i is then defined as the difference between the angles θ_p at the two neighbor pixels $i-k$ and $i+k$ along the edge of the image. At edge pixels located at the tips of leaves, such as a and c in fig. 4a, or at the corners of cut stem sections, such as b and d in fig. 4a, C_{urv} is always positive and will generally be much higher than at the rest of the edge pixels. Similarly C_{urv} is negative and high at branching sections, where leaves are connected to stems, and it is close to zero along the straight edges of stems.

Segmentation of the plantlet image into distinct blobs

The goal of the segmentation algorithm is to partition the plantlet image into distinct blobs such as A, B, C, D and E in fig. 4b. Then using characterizing features of "leaf blobs" and "stem blobs" identify each blob as a leaf or stem section.

The basic premise of the segmentation algorithm developed herein is that each blob can be delimited by two edge pixel chains, starting at points of maximal curvature, such as A1-A2, B1-B2, C1-C2, D1-D2 and E1-E2 in fig. 4b. The steps of the algorithm are as follows:

1. Scan the image and select a pixel with maximal curvature, i.e. where C_{urv} is larger than a preset threshold, provided that this pixel does not belong to a previously delimited blob. If no such pixel is found then stop.

Figure 3A. Example of a plantlet segment image, showing the tangent and normal of an edge pixel.

Figure 3B. Digitized part of fig. 3A, showing mode of computing curvature, width, and gray scale level along the normal of an edge pixel.

Figure 4A. Mode of segmenting plantlet image into distinct blobs, showing blob starting points, direction of growth and stopping sites.

Figure 4B. Image of plantlet piece, segmented into distinct blobs by pairs of edge pixel chains.

2. Starting from this pixel find two edge pixel chains which delimit a new blob, as illustrated by the arrows in fig. 4a. The blob growing process stops at branching sections or when a pixel of a previously defined blob is encountered, such as illustrated by the asterisks in fig. 4a. (Modus operandi of the blob growing process and stopping criterion will be described in the next section.)

3. Label the two edge pixel chains as belonging to the delimited blob.

4. Return to step 1 and repeat.

At the end of this process most of the plantlet has been mapped and labelled as blobs, such as A, B, C and D in fig. 4a. Relatively long sections of stems, such as the crosshatched stem section in fig. 4a, that does not have an external point of maximal curvature, are left unidentified. To find these "hidden" blob growth starting points proceed as follows:

5. Find adjacent edge pixels of labeled blobs, such as between blobs A and B or C and D, in fig. 4a.

6. Form clusters of blobs composed of blobs that have adjacent edge pixels found in 5. In fig. 4a blobs A and B can form one cluster while blobs D and C form another blob cluster.

7. Find adjacent edge pixels, if any, between the above blob clusters and edge pixels that have not yet been labeled. These are the hidden maximal curvature, blob growth starting points that can be used to form edge pixel chains which delimit the remaining blobs, just as in 2. In fig. 4b blob C was formulated in this manner.

8. Repeat until there are no unlabelled edge pixels left, whence the segmentation of the plantlet into distinctive blobs is complete.

In the next section we describe in detail the blob growing process and a novel "stopping criterion", which may be generally applied for stopping a region growing process at branching sights of a segmented image. Following that we shall use some characterizing features to classify each segmented blob as a leaf or stem section.

Blob growing process and stopping criterion

The blob growing process begins at edge pixels of maximal curvature, such as point A in fig. 5a, as described in the previous section. A single step of a blob growing process, e.g. from point B to point B_n is illustrated in fig. 5a, wherein all edge pixels from A to B and from A to C have already been found and mapped.

1. With pixel C as a pivot move along the opposite edge pixel chain, in the blob growing

Figure 5A. Demonstrating one step in blob growth process

Figure 5B. Step sequence in blob formation process, from the starting point of maximal curvature to the end at a branch intersection.

Figure 6. Examples of blob growth stopping at a branch. A. where a leaf is connected to a stem. B. where a stem is subdivided.

Figure 7. Typical cases of algorithm failure. A. Leaf with several points of high curvature. B. Leaf occluded by a branch. C. Short and narrow leaf may be classified as a stem. D. Short end of a stem may be classified as a leaf.

direction, while noting the width W from the inspected pixel to the pivot pixel C , as depicted by the arrows.

2. Stop moving when the width W begins to increase. In the example of fig. 5a the movement stopped at pixel B_n which becomes the new pivot pixel, whence the movement proceeds on the opposite edge pixel chain.

The result is alternate movements along the two edge sections of a blob, while maintaining minimal distances between the opposite edges of the growing blob, e.g. B_1-C_1 , B_2-C_2 , B_3-C_3 , etc. as depicted in fig. 5b. This is similar to the approach taken by Shapiro et.al (1981), for skeleton generation from X,Y boundary sequences.

TABLE 3

CLASSIFICATION RESULTS OF SECOND TEST SET

total segmentation failures	5.9%
misclassification of leaves	4.7%
misclassification of stems	1.1%
total misclassifications	11.7%

the total misclassification rate is 11.7%. Note that this is also a worst case scenario, because a plantlet segment can sometimes be correctly classified, even if there was a segmentation failure. This is an encouraging result, although there is considerable room for improvement before such a system can be used in practice.

Summary

A machine vision controlled automatic subculturing system for potato tissue cultures was presented, as a simpler and more cost effective solution, than the popular trend of imitating the manual subculturing task by a robot. A simple and relatively fast image processing algorithm was developed, particularly suitable for classification of potato tissue cultures.

In lieu of the general Medial Axis Transform approach, this specialized algorithm takes advantage of the inherent difference between the geometrical shape and gray scale levels of the stems and the leaves as well as on the rather simple connectivity rules of attachment between them. Visual inspection of the images of plantlet segments, in which the algorithm failed to correctly identify leaves and stems indicates some typical failure modes. Some examples of typical failures are shown in fig. 7. In case A the leaf contour has several points of high curvature, while in case B a leaf is occluded by a stem. These are typical situations that may cause segmentation failure. In case C and D the algorithm may fail to correctly classify a leaf or a stem, although the segmentation process was successful. Typically this happens when a short and narrow leaf is mistakenly classified as a stem, (case C), or when a short end of a stem is misclassified as a leaf, (case D).

There are several ways to remedy these problems, which we hope to investigate in the future. One obvious improvement, which is presented in chapter 6, is to use color imaging and exploit the color difference between leaves and stems as an additional robust feature, which is independent of the geometry. The classification accuracy may also be improved by using a better classifier, say a multi dimensional piecewise linear classifier, such as described by Sklansky and Michelotti 1980, in lieu of the simple assumption that all the features are normally distributed and orthogonal. Another approach is to use chemical, biological and environmental treatments to develop improved plantlet morphology, e.g. as uniform in size and shape as possible, which will also be suitable for automatic chopping into uniformly sized segments, on a conveying production line system.

In summary, these preliminary results indicate that machine inspection and classification of tissue culture plantlets is possible. In the next chapters some more aspects are presented, toward the development of an automatic system for mass micropropagation by tissue cultures.

REFERENCES

- ANDERSON, W.C., MEAGER, G.W. and NELSON, A.G. 1977. Hort. Sci. 12, 543.
- BRY, L. D. 1986. Robots in plant tissue culture: an insight. Newsletter International Association for Plant Tissue Culture (49):2-22
- CONGER, B.V. 1981. Cloning agricultural plants via in vitro techniques. CRC Press, Boca Raton, Fla. 1-273.
- DELEPLANQUE H., BONNET P. and POSTAIRE J. G. 1985. An Intelligent Robot for in vitro Plantlet Production. Proceedings of the 5th International Conference on Robot Vision and Sensory Controls ROVISEC 5 pp. 305-314.
- DUDA R. O. and HART P. E. 1973. Pattern Classification and Scene Analysis. J. Wiley and sons.
- HOLDGATE D. P. (1992). Automated Micropropagation and the Application of a Laser Beam for Cutting. To be presented at the International Symposium on Transplant Production Systems. Yokohama Japan July 21-26, 1992.
- KINASE A. and WATAKE H. 1991. Robot for Masspropagation. Proc. IFAC Mathematical and Control Applications in Agriculture and Horticulture. pp. 225-230.
- KUTZ L.J. 1986. Robotic transplanting. ASAE paper 86-1088.
- MIWA Y., YAMAMOTO T., KUSHIKASHI Y. and KODAMA H. 1988 Study on Automation of Plant Tissue Culture Process (1st Report) - Development of Automatic Seedling Transplantation System. J. Japan Soc. Precision Engin. (Japan) 54(6):1107-1112.
- MONGA O. 1987. An Optimal Region Growing Algorithm for Image Segmentation. International J. of Pattern Recognition and Artificial Intelligence 1(3&4):351:375.
- NIBACK C. W., CAPSON D. W. and GIBBONS P. B. (1990). Generating Skeletons and Centerlines from the Medial Axis Transform. Proc. 10th International Conference on Pattern Recognition. pp. 881-885.
- PAVLIDIS T. and YUH-TAY L. 1990. Integrating Region Growing and Edge Detection. IEEE

Transactions on Pattern Analysis and Machine Intelligence 12(3):225-233.

SIMONTON W. 1991. Automated Plant Handling and Processing in a Robotic Workcell. Proc. IFAC Mathematical and Control Applications in Agriculture and Horticulture. pp. 213-218.

SHAPIRO B. PISA J. AND SKLANSKY J. 1981. Skeleton Generation from X,Y Boundary Sequences. Computer Graphics and Image Processing 15:136-153.

SKLANSKY J. and L. MICHELOTTI. 1980. Locally Trained Piecewise Linear Classifier. IEEE Trans. on Pattern Analysis and Machine Intelligence, Vol: PAMI-2, No. 2: 101-111.

SKLANSKY J. 1974. On Filling Cellular Concavities. Computer Graphics and Image Processing 4:236-247.

SLUIS C. J. 1985. Commercialization of plant tissue culture propagation. Newsletter International Association for Plant Tissue Culture (47):2-12.

ROSENFELD A. AND KAK A. C. 1982 Digital Picture Processing Vol. 2 2nd.ed. Academic Press.

ROW J. W. 1986. Tissue Culture as a plant Production System for Horticultural Crops. pp 35-45. Martinus Nijhoff Publishers. (Edited by Zimmerman R. H. et al.)

WALKER, K.A. 1986. Automation of *in vitro* plant propagation: Has its time finally arrived? Genetic Engineering News, July/August:52

WATAKE H. and KINASE A. 1988 Robot for Tissue Culture. Robot (Japan) 64:74-79.

Chapter 5 COLORED ILLUMINATION FOR ENHANCING DISCRIMINABILITY IN MACHINE VISION

Introduction

How an object appears to a machine vision system can be significantly affected by the illumination, the image sensors, and the scene geometry[3]. By carefully choosing illuminations and sensors and by configuring them appropriately with respect to the objects in the scene, many visual inspection tasks can be automated to achieve high speed of inspection and low cost.

In this part we show how to design a colored illumination that maximizes the contrast between two objects in red-green-blue (RGB) images. By carefully choosing colored illumination and by configuring the sources appropriately with respect to the object being inspected, we can often achieve higher speeds and lower cost as well as improved accuracy of segmentation of objects and materials in the image. We tested our design technique successfully on painted color patches and a live potato plant.

Our technique uses knowledge of the camera's spectral response curves and the object's spectral reflectance to find an optimal colored illumination. An object's spectral reflectance $s(\lambda)$ denotes the ratio of the intensities of the reflected and incident light on an object as a function of wavelength λ . Figure 1 illustrates two spectral reflectance curves $s_a(\lambda)$ and $s_b(\lambda)$ for two hypothetical objects 'a' and 'b'. The difference between the two spectral response curves, $s_a(\lambda) - s_b(\lambda)$ is a measure of the discriminability between 'a' and 'b', since the objects can not be discriminated by their reflectances if $s_a(\lambda) = s_b(\lambda)$. The discriminability also depends on the illumination $i(\lambda)$ (intensity versus wavelength), since the objects cannot be discriminated by machine vision if $i(\lambda)=0$ for all λ . The discriminability also depends on the red, green, and blue spectral response of the camera, $f_r(\lambda)$, $f_g(\lambda)$, $f_b(\lambda)$, since the objects cannot be discriminated if these responses are zero for all λ . The spectral responses for our color CCD camera are shown in Figure 2. Thus the following three functions are measures of the discriminability of the objects

by each of the three color sensors:

$$f_R(\lambda) i(\lambda) [s_o(\lambda) - s_b(\lambda)] \quad (1-1)$$

$$f_G(\lambda) i(\lambda) [s_o(\lambda) - s_b(\lambda)] \quad (1-2)$$

$$f_B(\lambda) i(\lambda) [s_o(\lambda) - s_b(\lambda)] \quad (1-3)$$

These curves are illustrated in Figure 3 for $i(\lambda) = 1$. For a given illumination energy

$$\int_{-\infty}^{\infty} i(\lambda) d\lambda \quad (1-4)$$

the discriminability by these sensors is maximized by choosing $i(\lambda)$ such that the integrals with respect to λ of Equations (1-1) - (1-3) are maximized. This usually results in a colored illumination whose $i(\lambda)$ has peaks at wavelengths where Equations (1-1) - (1-3) are maximum. Figure 4 illustrates an illumination spectrum $i(\lambda)$ that might be used to enhance the discriminability between 'a' and 'b'. The technique we used to determine this colored illumination is described in Section 5.

Figure 1 - Spectral reflectance curves for simulated objects 'a' and 'b'.

Figure 2 - Spectral response of our color CCD camera in the R, G, and B channels.

Figure 3 - Zeta curves indicating the measurable contrast between objects 'a' and 'b' by the three color sensors.

Figure 4 - A contrast enhancing illumination spectrum.

The Camera Model

In this section, we present a mathematical model of the formation of RGB images. We cover the visible spectrum by three overlapping color bands, labeled R, G, and B (for "red", "green", and "blue"). Each color band corresponds to the range of wavelengths associated with one of the three color sensors in a color video camera. We denote band R by $[\lambda_{R0}, \lambda_{R1}]$, the band G by $[\lambda_{G0}, \lambda_{G1}]$, and the band B by $[\lambda_{B0}, \lambda_{B1}]$. In our camera, the band R is $[540\text{nm}, 700\text{nm}]$, the band G is $[390\text{nm}, 590\text{nm}]$, and the band B is $[300\text{nm}, 440\text{nm}]$.

Suppose $f(x,y)$ is a color image with pixels p_i defined by $p_i = (R_i, G_i, B_i)$. The process by which the camera produces pixel values for bands R, G, B is modeled by the following *spectral integration equations*:

$$R_i = S_{Ri} + n(R_i) \quad (2-1)$$

$$G_i = S_{Gi} + n(G_i) \quad (2-2)$$

$$B_i = S_{Bi} + n(B_i) \quad (2-3)$$

where

$$S_{Ri} = \alpha_R \int_{\lambda_{R0}}^{\lambda_{R1}} i(\lambda) s_f(\lambda) f_R(\lambda) d\lambda \quad (2-4)$$

$$S_{G_i} = \alpha_G \int_{\lambda_{G_0}}^{\lambda_{G_1}} i(\lambda) s_i(\lambda) f_G(\lambda) d\lambda \quad (2-5)$$

$$S_{B_i} = \alpha_B \int_{\lambda_{B_0}}^{\lambda_{B_1}} i(\lambda) s_i(\lambda) f_B(\lambda) d\lambda \quad (2-6)$$

and where $i(\lambda)$ is the illumination power spectrum, $s(\lambda)$ is the spectral reflectance of the object in the direction of the camera, and $f_R(\lambda)$, $f_G(\lambda)$, and $f_B(\lambda)$ are the camera's spectral response functions in the R, G, and B bands. The terms $n(R_i)$, $n(G_i)$, and $n(B_i)$ are additive camera noise in the R, G, and B channels. A study of radiometric noise estimation was performed by Healey [2] and provides a useful camera noise model for our work. The constants α_R , α_G , and α_B are gain factors for the R, G, and B camera channels. These gains are determined so that the camera produces its maximum output in each channel when a white object ($s(\lambda) = 1.0$) is viewed under a maximum intensity white light ($i(\lambda) = I_{\max}$):

$$\alpha_R = \frac{R_{\max}}{I_{\max} \int_{\lambda_{R_0}}^{\lambda_{R_1}} f_R(\lambda) d\lambda} \quad (2-7)$$

$$\alpha_G = \frac{G_{\max}}{I_{\max} \int_{\lambda_{G_0}}^{\lambda_{G_1}} f_G(\lambda) d\lambda} \quad (2-8)$$

$$\alpha_B = \frac{B_{\max}}{I_{\max} \int_{\lambda_{B_0}}^{\lambda_{B_1}} f_B(\lambda) d\lambda} \quad (2-9)$$

The set of all ordered triples (R_i, G_i, B_i) that can be produced by an imaging system defines *RGB space*.

When a diffusely reflecting object, having nonzero reflectance in the R, G, and B color bands, is illuminated with white light, the camera produces nonzero R_i , G_i , and B_i for those pixels in the image corresponding to the object. When these pixels are mapped into RGB space, pixels that are similar in color and brightness form a *cluster*. We estimate the discriminability of objects by measuring distances among the clusters formed by these objects in RGB space.

The Mahalanobis Distance Metric

In this section, we describe a metric for quantifying interobject contrast via cluster discriminability. The variance of a cluster formed by pixels corresponding to a single object can increase in the presence of camera noise and pixel-to-pixel variations in spectral reflectance. This increase in cluster variance can cause otherwise distinct clusters to overlap and by that decreases the discriminability between the clusters and the interobject contrast. Thus, it is necessary that our metric for interobject contrast accounts for the variance of the object clusters in RGB space.

The Mahalanobis distance measures cluster discriminability subject to the constraint that the clusters are Gaussian distributed and have the same covariance matrix Σ . In addition, the Mahalanobis distance accounts for the correlations among the $\{R_i, G_i, B_i\}$. The Mahalanobis distance between two clusters C_a and C_b is defined by

$$d(C_a, C_b) = \sqrt{(\mu_a - \mu_b)' (\Sigma)^{-1} (\mu_a - \mu_b)} \quad (3-1)$$

where the cluster means μ_a and μ_b are defined by

$$\mu_a = E \begin{bmatrix} R_{a1} \\ G_{a1} \\ B_{a1} \end{bmatrix} = \begin{bmatrix} \overline{R_a} \\ \overline{G_a} \\ \overline{B_a} \end{bmatrix} \quad (3-2)$$

$$\mu_b = E \begin{bmatrix} R_{b1} \\ G_{b1} \\ B_{b1} \end{bmatrix} = \begin{bmatrix} \overline{R_b} \\ \overline{G_b} \\ \overline{B_b} \end{bmatrix} \quad (3-3)$$

where $E[\cdot]$ denotes statistical expectation and $\overline{X} = E[X]$. The covariance matrix Σ for clusters C_a and C_b is

$$\Sigma = \begin{bmatrix} \Sigma_{RR} & \Sigma_{RG} & \Sigma_{RB} \\ \Sigma_{GR} & \Sigma_{GG} & \Sigma_{GB} \\ \Sigma_{BR} & \Sigma_{BG} & \Sigma_{BB} \end{bmatrix} \quad (3-4)$$

where the variances and the covariances in Equation (3-4) are defined by

$$\sigma_R^2 = \Sigma_{RR} = E[(R_{a1} - \overline{R_a})^2] = E[(R_{b1} - \overline{R_b})^2] \quad (3-5)$$

$$\sigma_G^2 = \Sigma_{GG} = E[(G_{a1} - \overline{G_a})^2] = E[(G_{b1} - \overline{G_b})^2] \quad (3-6)$$

$$\sigma_B^2 = \Sigma_{BB} = E[(B_{a1} - \overline{B_a})^2] = E[(B_{b1} - \overline{B_b})^2] \quad (3-7)$$

$$\Sigma_{RG} = \Sigma_{GR} = E[(R_{a1} - \overline{R_a})(G_{a1} - \overline{G_a})] = E[(R_{b1} - \overline{R_b})(G_{b1} - \overline{G_b})] \quad (3-8)$$

$$\Sigma_{RB} = \Sigma_{BR} = E[(R_{a1} - \overline{R_a})(B_{a1} - \overline{B_a})] = E[(R_{b1} - \overline{R_b})(B_{b1} - \overline{B_b})] \quad (3-9)$$

$$\Sigma_{GB} = \Sigma_{BG} = E[(G_{a1} - \overline{G_a})(B_{a1} - \overline{B_a})] = E[(G_{b1} - \overline{G_b})(B_{b1} - \overline{B_b})] \quad (3-10)$$

The covariances in Equations (3-8)-(3-10) are generally nonzero due to overlap in the camera's spectral response curves; and, thus Σ is usually not diagonal. Substituting Equations (3-2)-(3-10) into Equation (3-1) yields an expanded form of the Mahalanobis distance metric that is useful for measuring cluster discriminability in RGB space:

$$d(C_a, C_b) = \sqrt{\begin{bmatrix} \overline{R_a} - \overline{R_b} \\ \overline{G_a} - \overline{G_b} \\ \overline{B_a} - \overline{B_b} \end{bmatrix}^T \begin{bmatrix} \sigma_R^2 & \sigma_{RG} & \sigma_{RB} \\ \sigma_{GR} & \sigma_G^2 & \sigma_{GB} \\ \sigma_{BR} & \sigma_{BG} & \sigma_B^2 \end{bmatrix}^{-1} \begin{bmatrix} \overline{R_a} - \overline{R_b} \\ \overline{G_a} - \overline{G_b} \\ \overline{B_a} - \overline{B_b} \end{bmatrix}} \quad (3-11)$$

Our objective in the next section is to devise a colored illumination $i(\lambda)$, using the image formation model in Section 2, which maximizes the Mahalanobis distance given by Equation (3-11).

Optimal Colored Illumination from Spectral Reflectance

In this section we describe how to design a colored illumination that maximizes the Mahalanobis distance between a pair of objects. We begin by expressing the Mahalanobis distance, Equation (3-11), in terms of the illumination power spectrum, the camera spectral response, and the spectral reflectance of the objects in the scene. Based on this formula, we develop a technique for designing a colored illumination that maximizes the discriminability between objects in the R, G, and B camera channels.

The differences between the means in Equation (3-11) are given by the *color difference equations*

$$\overline{R}_o - \overline{R}_b = \int i(\lambda) f_R(\lambda) [\overline{s}_o(\lambda) - \overline{s}_b(\lambda)] d\lambda \quad (4-1)$$

$$\overline{G}_o - \overline{G}_b = \int i(\lambda) f_G(\lambda) [\overline{s}_o(\lambda) - \overline{s}_b(\lambda)] d\lambda \quad (4-2)$$

$$\overline{B}_o - \overline{B}_b = \int i(\lambda) f_B(\lambda) [\overline{s}_o(\lambda) - \overline{s}_b(\lambda)] d\lambda \quad (4-3)$$

In Equations (4-1)-(4-3), the products of the camera spectral response and the difference between the spectral reflectance curves are determined by the properties of the camera and the objects in the scene. Because neither the camera spectral response nor the spectral reflectance properties of the objects are parameters that can be manipulated to increase the contrast between the objects, we group these fixed parameters together to form the *zeta curves*

$$\zeta_R(\lambda) = f_R(\lambda) [\overline{s}_o(\lambda) - \overline{s}_b(\lambda)] \quad (4-4)$$

$$\zeta_G(\lambda) = f_G(\lambda) [\overline{s}_o(\lambda) - \overline{s}_b(\lambda)] \quad (4-5)$$

$$\zeta_B(\lambda) = f_B(\lambda) [\overline{s}_o(\lambda) - \overline{s}_b(\lambda)] \quad (4-6)$$

The maxima and minima of the zeta curves correspond to wavelengths λ at which the camera senses large contrast between the two objects. Substituting Equations (4-4)-(4-6) into Equations (4-1)-(4-3), respectively, results in a simplified set of color difference equations

$$\overline{R}_o - \overline{R}_b = \int i(\lambda) \zeta_R(\lambda) d\lambda \quad (4-7)$$

$$\overline{G}_o - \overline{G}_b = \int i(\lambda) \zeta_G(\lambda) d\lambda \quad (4-8)$$

$$\overline{B}_o - \overline{B}_b = \int i(\lambda) \zeta_B(\lambda) d\lambda \quad (4-9)$$

A detailed derivation of Equations (4-7)-(4-9) is presented in Appendix A.1. It is convenient to interpret Equations (4-7)-(4-9) as a weighted sum of the area under the zeta curves, $\zeta_R(\lambda)$, $\zeta_G(\lambda)$, and $\zeta_B(\lambda)$, where $i(\lambda)$ is a wavelength dependent weighting function.

The variances of the R, G, and B signals in the camera are given by the *color variance equations*

$$\sigma_R^2 = v_R^2 + w_R^2 \quad (4-10)$$

$$\sigma_G^2 = v_G^2 + w_G^2 \quad (4-11)$$

$$\sigma_B^2 = v_B^2 + w_B^2 \quad (4-12)$$

where the variances of the noise-free R, G, and B camera values are

$$v_R^2 = E[(S_R)^2] - \bar{R}^2 \quad (4-13)$$

$$v_G^2 = E[(S_G)^2] - \bar{G}^2 \quad (4-14)$$

$$v_B^2 = E[(S_B)^2] - \bar{B}^2 \quad (4-15)$$

and the variances of the zero-mean camera noise in the R, G, and B camera channels are

$$w_R^2 = E[n(R)^2] \quad (4-16)$$

$$w_G^2 = E[n(G)^2] \quad (4-17)$$

$$w_B^2 = E[n(B)^2] \quad (4-18)$$

A detailed derivation of Equations (4-16)-(4-18) is presented in Appendix A.2.

When the camera's spectral response curves overlap, the R_i , G_i , and B_i may be correlated. The covariances of R_i , G_i , and B_i are given by the *color covariance equations*

$$\Sigma_{RG} = E[(R_i - \bar{R})(G_i - \bar{G})] \quad (4-19)$$

$$\Sigma_{RB} = E[(R_i - \bar{R})(B_i - \bar{B})] \quad (4-20)$$

$$\Sigma_{GB} = E[(G_i - \bar{G})(B_i - \bar{B})] \quad (4-21)$$

If the camera noise sources are uncorrelated, and the camera noise and RGB signals are uncorrelated, then Equations (4-19)-(4-21) may be expressed as

$$\Sigma_{RG} = E \left[\int i(\lambda_1) f_R(\lambda_1) [s_R(\lambda_1) - \bar{s}(\lambda_1)] d\lambda_1 \int i(\lambda_2) f_G(\lambda_2) [s_G(\lambda_2) - \bar{s}(\lambda_2)] d\lambda_2 \right] \quad (4-22)$$

$$\Sigma_{RB} = E \left[\int i(\lambda_1) f_R(\lambda_1) [s_R(\lambda_1) - \bar{s}(\lambda_1)] d\lambda_1 \int i(\lambda_2) f_B(\lambda_2) [s_B(\lambda_2) - \bar{s}(\lambda_2)] d\lambda_2 \right] \quad (4-23)$$

$$\Sigma_{GB} = E \left[\int i(\lambda_1) f_G(\lambda_1) [s_G(\lambda_1) - \bar{s}(\lambda_1)] d\lambda_1 \int i(\lambda_2) f_B(\lambda_2) [s_B(\lambda_2) - \bar{s}(\lambda_2)] d\lambda_2 \right] \quad (4-24)$$

A detailed derivation of Equations (4-22)-(4-24) is presented in Appendix A.3. The color difference equations, Equations (4-7)-(4-9), color variance equations, Equations (4-10)-(4-18), and color covariance equations, Equations (4-22)-(4-24), can be substituted into Equation (3-11) to express the Mahalanobis distance as a function of the illumination $i(\lambda)$.

To maximize the Mahalanobis distance, we define a *parametric illumination model*, and we search the multidimensional illumination space for the best contrast enhancing illumination. This optimal illumination can be found by gradient descent.

In the next section we present a parametric illumination model, called *multigaussian spectrum illumination*. This illumination model is useful for the design of broad-band colored illuminations that can be implemented using broad-band color filters.

Optimal Multigaussian Spectrum Illumination

In this section we show how to find an optimal colored illumination formed by summing three Gaussian spectra. We refer to this as *multigaussian spectrum illumination*. We are interested in multigaussian spectrum illuminations since most color filters used to color an illumination have broad-band, nearly Gaussian spectral transmittance curves. In the parametric illumination model discussed this section, the illumination spectrum $i(\lambda)$ is a sum of three Gaussian spectra having means at λ_R , λ_G , and λ_B , variances σ_{IR}^2 , σ_{IG}^2 , and σ_{IB}^2 , and scale factors i_R , i_G , and i_B :

$$i(\lambda) = i_R N(\lambda_R, \sigma_R) + i_G N(\lambda_G, \sigma_G) + i_B N(\lambda_B, \sigma_B) \quad (5-1)$$

where

$$N(\lambda, \sigma) = \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{(\lambda - \lambda_x)^2}{2\sigma_x^2}} \quad \forall x \in \{R, G, B\}. \quad (5-2)$$

Substituting Equation (5-1) into Equations (4-1)-(4-3) yields

$$\overline{R_o} - \overline{R_b} = i_R \int N(\lambda_R, \sigma_{IR}) \zeta_R(\lambda) d\lambda + i_G \int N(\lambda_G, \sigma_{IG}) \zeta_R(\lambda) d\lambda + i_B \int N(\lambda_B, \sigma_{IB}) \zeta_R(\lambda) d\lambda \quad (5-3)$$

$$\overline{G_o} - \overline{G_b} = i_R \int N(\lambda_R, \sigma_{IR}) \zeta_G(\lambda) d\lambda + i_G \int N(\lambda_G, \sigma_{IG}) \zeta_G(\lambda) d\lambda + i_B \int N(\lambda_B, \sigma_{IB}) \zeta_G(\lambda) d\lambda \quad (5-4)$$

$$\overline{B_o} - \overline{B_b} = i_R \int N(\lambda_R, \sigma_{IR}) \zeta_B(\lambda) d\lambda + i_G \int N(\lambda_G, \sigma_{IG}) \zeta_B(\lambda) d\lambda + i_B \int N(\lambda_B, \sigma_{IB}) \zeta_B(\lambda) d\lambda. \quad (5-5)$$

Equations (5-3)-(5-5) show that each multigaussian spectrum light source may contribute to the color difference in each color band. This is because each of the component Gaussian illuminations in Equation (5-1) can overlap $\zeta_R(\lambda)$, $\zeta_G(\lambda)$, and $\zeta_B(\lambda)$ thereby contributing to the luminance values measured in the R, G, and B camera channels, respectively.

Substituting Equation (5-1) into Equations (4-10)-(4-18) yields color variance equations for multigaussian spectrum illuminants. Specifically, substituting (5-1) into Equation (4-13) yields

$$v_R^2 = E[(S_R)^2] - \overline{R}^2 \quad (5-6)$$

where

$$E[(S_R)^2] = E[i_R \int g_R(\lambda, \sigma_{IR}^2) f_R(\lambda) s(\lambda) d\lambda + i_G \int g_G(\lambda, \sigma_{IG}^2) f_R(\lambda) s(\lambda) d\lambda + i_B \int g_B(\lambda, \sigma_{IB}^2) f_R(\lambda) s(\lambda) d\lambda] \quad (5-7)$$

and

$$\overline{R}^2 = \left(E[i_R \int g_R(\lambda, \sigma_{IR}^2) f_R(\lambda) \overline{s}(\lambda) d\lambda + i_G \int g_G(\lambda, \sigma_{IG}^2) f_R(\lambda) \overline{s}(\lambda) d\lambda + i_B \int g_B(\lambda, \sigma_{IB}^2) f_R(\lambda) \overline{s}(\lambda) d\lambda] \right)^2 \quad (5-8)$$

Substituting Equation (5-1) into Equation (4-14) yields

$$v_G^2 = E[(S_G)^2] - \overline{G}^2 \quad (5-9)$$

where

$$E[(S_G)^2] = E[i_R \int g_R(\lambda, \sigma_{IR}^2) f_G(\lambda) s(\lambda) d\lambda + i_G \int g_G(\lambda, \sigma_{IG}^2) f_G(\lambda) s(\lambda) d\lambda + i_B \int g_B(\lambda, \sigma_{IB}^2) f_G(\lambda) s(\lambda) d\lambda] \quad (5-10)$$

and

$$\overline{G}^2 = \left(E[i_R \int g_R(\lambda) \sigma_{IR}^2 f_G(\lambda) \overline{s}_R(\lambda) d\lambda + i_G \int g_G(\lambda) \sigma_{IG}^2 f_G(\lambda) \overline{s}_G(\lambda) d\lambda + i_B \int g_B(\lambda) \sigma_{IB}^2 f_G(\lambda) \overline{s}_B(\lambda) d\lambda] \right)^2 \quad (5-11)$$

Finally, substituting Equation (5-1) into Equation (4-15) yields

$$v_B^2 = E[(S_B)^2] - \overline{B}^2 \quad (5-12)$$

where

$$E[(S_B)^2] = E[i_R \int g_R(\lambda) \sigma_{IR}^2 f_B(\lambda) s_B(\lambda) d\lambda + i_G \int g_G(\lambda) \sigma_{IG}^2 f_B(\lambda) s_B(\lambda) d\lambda + i_B \int g_B(\lambda) \sigma_{IB}^2 f_B(\lambda) s_B(\lambda) d\lambda] \quad (5-13)$$

and

$$\overline{B}^2 = \left(E[i_R \int g_R(\lambda) \sigma_{IR}^2 f_B(\lambda) \overline{s}_B(\lambda) d\lambda + i_G \int g_G(\lambda) \sigma_{IG}^2 f_B(\lambda) \overline{s}_B(\lambda) d\lambda + i_B \int g_B(\lambda) \sigma_{IB}^2 f_B(\lambda) \overline{s}_B(\lambda) d\lambda] \right)^2 \quad (5-14)$$

Equations (5-6)-(5-14) describe the variance of the R, G, and B values for a multigaussian spectrum illumination. Similarly, Equation (5-1) can be substituted into Equations (4-22)-(4-24) to yield color covariance equations for multigaussian spectrum illuminations. By substituting the color difference equations, Equations (5-3)-(5-5), the color variance equations, Equations (5-6)-(5-14), and the appropriate color covariance equations into Equation (3-11), the Mahalanobis distance can be expressed as a function of the multigaussian illumination $i(\lambda)$. To find a contrast enhancing colored illumination, we use a variable step gradient descent algorithm [4] to search the nine-dimensional parametric illumination space for an illumination vector that maximizes this Mahalanobis distance. The nine components of the illumination vectors in this space are $\{i_R, i_G, i_B, \lambda_R, \lambda_G, \lambda_B, \sigma_{IR}, \sigma_{IG}, \sigma_{IB}\}$.

An Experiment using Simulated Illumination

In this section, we present the results of an experiment in which we simulated colored illuminations that enhances the discriminability of pairs of painted color patches on a Macbeth ColorChecker color chart. In this experiment, a gradient descent algorithm was used to find an optimal multigaussian spectrum illumination and to determine an optimal combination of Wratten gel filters for simulating multigaussian spectrum illuminations. Because the center wavelengths of the Wratten gel filters are fixed, the parametric illumination model for the second gradient descent search consists of three illumination intensities and three color filters: $(i_1, i_2, i_3, W_1, W_2, W_3)$. Upon finding an optimal set of color filters (W_1, W_2, W_3) and the appropriate illumination intensities (i_1, i_2, i_3) , we simulated the colored illumination and measured the resulting Mahalanobis distance. In our experiment, the discovered optimal colored illuminations produced higher interobject discriminability than that produced with white light.

Our machine vision workstation uses three IBM-PC compatible image processing boards: an RGB/HSI Color Frame Grabber, an Auxiliary Frame Processor, and a Video Decoder/Encoder. In addition, a Sony XC-711 color (RGB/NTSC) CCD video camera and a Sony Trinitron color monitor generate and display color images, respectively. In addition, we constructed a lighting chamber in which we conducted our illumination experiments. The lighting chamber consists of six Sylvania fluorescent white lights mounted inside a cabinet with a mirrored interior. A color CCD camera is mounted at the top-center of the cabinet so that the field of vision is directly beneath the camera. Crumpled aluminum foil is used to line the sides of the lighting chamber to create a more diffuse illumination and a backlit stage supports the objects in the scene.

Figure 5 – Schematic description of light cabinet

In our experiment, colored illuminations were simulated by forming an appropriately weighted sum of basis images:

$$f(x,y) = i_1 f_{w_1}(x,y) + i_2 f_{w_2}(x,y) + i_3 f_{w_3}(x,y) \quad (6-1)$$

where (i_1, i_2, i_3) is the illumination intensity vector and $(f_{w_1}(x,y), f_{w_2}(x,y), f_{w_3}(x,y))$ are the three basis images obtained using Wratten filters (W_1, W_2, W_3) , respectively. The set of basis images $\{W_i\}$ was acquired by placing Kodak Wratten gel filters in front of the camera lens to simulate the effects of colored illumination. The camera aperture and illumination intensity were not adjusted during the acquisition of the basis image set. The Wratten gel filters used in our experiments were: #22, #24, #29, #44, #47B, #50, #58, #90, and #99 [5]. Figure 6 illustrates the spectral transmission curves for these filters.

Figure 6 - Spectral transmission curves of the Wratten gel filters.

We considered the problem of enhancing the contrast between pairs of painted color patches on a Macbeth color chart[6]. Using the spectral reflectance curves for the color patches and the camera's spectral response curves, we found a contrast enhancing multigaussian spectrum illumination and the combination of Wratten gel filters that best approximates this illumination. We then constructed an image $f(x,y)$ by appropriately scaling and summing the three basis images corresponding to the selected Wratten gel filters as described by Equation (6-1). The Mahalanobis distance was computed for the objects in $f(x,y)$ and plotted as a function of illumination intensity for comparison with other lighting conditions.

Figure 7 illustrates the measured Mahalanobis distance for a red and a green pair of color patches using four distinct filters representing four distinct illuminations: WHITE, RED, RED-GREEN, and RED-GREEN-BLUE. The use of capital letters to denote the filters is a reminder that they represent broad band spectra. These results suggest that RED light (Wratten gel filter #24) can be used to enhance the measured contrast for low intensity images. This illumination is useful when the total illumination power is limited or the objects in the scene are photo sensitive. The curve for RED light ends at an intensity value of 54 due to camera saturation in the R camera channel. RED-GREEN light (Wratten gel Filters #24 & #58) can be used to enhance the measured contrast for moderate intensity images. Notice that this illumination produces a maximum Mahalanobis distance that is larger than that for white light and uses less illumination power. Finally, RED-GREEN-BLUE light (Wratten gel Filters #24, #58, & #50) can be used to enhance the measured contrast for high intensity images.

Figure 7 - Plot of Mahalanobis Distance vs. Image Intensity for a red-green of color patches.

Our results illustrate that the contrast between colored surfaces depends strongly on the properties of the objects and the illumination. Additionally, our results suggest that an appropriately designed colored illumination is superior to white illumination for discriminating pairs of differently colored patches.

Approximation Techniques for Real Illumination

Since our technique for designing colored illumination requires an estimate of the spectral reflectances of the objects, and since finding these spectral reflectances usually requires the use of expensive equipment (a photodensitometer), we developed simplified approximations to the spectral reflectances and to the parametric illumination model. We estimated the spectral reflectance as piecewise constant function — constant in each of three nonoverlapping bands matched to the camera's R, G, and B sensors — and the parametric illumination model as a triple line spectrum having one line in each of the three color bands. This approach permitted us to determine a triple line spectrum that maximized the Mahalanobis distance between the two classes of objects.

We tested this approach on a large assortment of genetically similar potato plants. Figure 8 illustrates a typical potato plant. In this experiment, the spectral reflectance of the leaves and the stem were unknown. To estimate the spectral reflectances, we compared the leaf and stem colors for several plants to the colors in a Munsell color chart. Using the spectral reflectance curves for the corresponding Munsell colors, we determined that RED-GREEN-BLUE light having the intensity ratio $(i_R, i_G, i_B) = (1:1.15:2.5)$ enhances the contrast between the leaves and the stem. This result enabled us to choose the best colored illumination from a set of sixteen available in our laboratory; each of which were calibrated on a color neutral patch in a Macbeth ColorChecker color chart. We confirmed the validity of this choice by finding the Mahalanobis distances among the two clusters of digitized R, G, and B signals associated with the leaves and stems. This test supported the effectiveness of our approximation technique and it showed that colored illumination can be more effective than white light in discriminating leaves and stems by machine vision. The details of this experiment and the associated approximation techniques are given in Technical Reports TP-91-3 [8] and TP-92-X [9] and in [10].

Figure 8 - A typical potato plant.

Conclusion

We presented a technique for selecting the best colored illumination condition for maximizing the contrast between objects when the spectral reflectance $s(\lambda)$ of the objects in the scene and the camera's spectral response curves are known. The experiments described in Section 6 confirm the effectiveness of our technique for finding colored illuminations that maximize contrast between objects in the scene. Our results suggest that an appropriately designed colored illumination is superior to white illumination for discriminating differently colored objects.

Appendix A -

In this appendix, we derive the *color difference equations*, the *color variance equations*, and the *color covariance equations* used in Section 4. Because each of these sets of equations involves repetitive derivations for each of the R, G, and B color bands, we present only the derivations for the R color band. The derivations for the G and B color bands follow a development that is parallel to that of the R color band. This appendix is divided into three sections: Section A.1 presents the derivation of the color difference equation for the R color band, Section A.2 we presents the derivation for the color variance equation for the R color band, and Section A.3 presents the derivation for the color covariance equations for the R color band.

A.1 Color Difference Equations.

Suppose that the red camera values for objects 'a' and 'b' are effectively modeled by the spectral integration equations:

$$R_{ai} = S_{Rai} + n(R_{ai}) \quad (A-1)$$

$$R_{bi} = S_{Rbi} + n(R_{bi}) \quad (A-2)$$

where

$$S_{Rai} = \alpha_R \int_{\lambda_{R0}}^{\lambda_{R1}} i(\lambda) s_{ai}(\lambda) f_R(\lambda) d\lambda \quad (A-3)$$

$$S_{Rbi} = \alpha_R \int_{\lambda_{R0}}^{\lambda_{R1}} i(\lambda) s_{bi}(\lambda) f_R(\lambda) d\lambda \quad (A-4)$$

and S_{Rai} and S_{Rbi} are two noise-free signals (instances of S_{Ri}) produced by the camera for objects 'a' and 'b'. We assume that the noise for the R camera channel is unbiased

$$\overline{n_R} = E[n(R_i)] = 0, \quad (A-5)$$

has a nonzero variance

$$\sigma^2(R) = E[n(R_i)^2] \neq 0, \quad (A-6)$$

and is uncorrelated with the signal S_{Ri}

$$E[S_{Ri} n(R_i)] = 0. \quad (A-7)$$

The expected red value of the pixels corresponding to object 'a' and 'b' is given by

$$\overline{R}_a = E[R_a] = \int i(\lambda) f_R(\lambda) \overline{s}_a(\lambda) d\lambda \quad (A-8)$$

$$\overline{R}_b = E[R_b] = \int i(\lambda) f_R(\lambda) \overline{s}_b(\lambda) d\lambda \quad (A-9)$$

where $\overline{s}_a(\lambda)$ and $\overline{s}_b(\lambda)$ are the mean spectral reflectance curves for objects 'a' and 'b'. The color difference equation, defined by the difference between Equations (A-8) and (A-9), provides a measure of the contrast between objects 'a' and 'b' under an illumination $i(\lambda)$:

$$\overline{R}_a - \overline{R}_b = \int i(\lambda) f_R(\lambda) [\overline{s}_a(\lambda) - \overline{s}_b(\lambda)] d\lambda = \int i(\lambda) \zeta_R(\lambda) d\lambda \quad (A-10)$$

Analogous equations can be derived for the color differences in the G and B camera channels.

A.2 Color Variance Equations.

To use the Mahalanobis distance for measuring the discriminability between clusters C_a and C_b , the variance of the red pixel values R_a and R_b must be equal:

$$\sigma_R^2 = \sigma^2(R_a) = \sigma^2(R_b) \quad (A-11)$$

Using the pixels from object 'a', the variance of the red pixel values is

$$\sigma_R^2 = E[(R_a - \overline{R}_a)^2] = E[R_a^2] - \overline{R}_a^2 \quad (A-12)$$

Substituting Equations (A-1) and (A-3) for R_a , and Equation (C-8) for $\overline{R}_a$ into Equation (A-12) and simplifying the resulting expression yields

$$\sigma_R^2 = v^2(R) + w^2(R) \quad (A-13)$$

where

$$v_R^2 = E[S_{Rad}^2] - \overline{R}_a^2 \quad (A-14)$$

is the noise-free variance of the red pixel values and where

$$w_R^2 = E[n(R_a)^2] \quad (A-15)$$

is the variance of the noise in the R camera channel. Analogous equations can be derived for the color variances in the G and B camera channels.

A.3 Color Covariance Equations.

When the camera's spectral response curves overlap along the wavelength axis, the R, G, and B pixels values are correlated. The covariance of the R and G pixel values is

$$\Sigma_{RG} = E[(R_i - \overline{R})(G_i - \overline{G})] \quad (A-16)$$

where R_i , $\overline{R}$, G_i , and $\overline{G}$ are given by

$$R_i = \alpha_R \int_{\lambda_{R0}}^{\lambda_{R1}} i(\lambda) s_i(\lambda) f_R(\lambda) d\lambda + n(R_i) \quad (A-17)$$

$$\bar{R} = \alpha_R \int_{\lambda_{R0}}^{\lambda_{R1}} i(\lambda) \bar{s}(\lambda) f_R(\lambda) d\lambda \quad (A-18)$$

$$G_i = \alpha_G \int_{\lambda_{G0}}^{\lambda_{G1}} i(\lambda) s_i(\lambda) f_G(\lambda) d\lambda + n(G_i) \quad (A-19)$$

$$\bar{G} = \alpha_G \int_{\lambda_{G0}}^{\lambda_{G1}} i(\lambda) \bar{s}(\lambda) f_G(\lambda) d\lambda . \quad (A-20)$$

By substituting Equations (A-17) and (A-20) into Equation (A-16) and by assuming that the camera noise sources $n(R_i)$ and $n(G_i)$ are uncorrelated,

$$E[n(R_i) n(G_i)] = 0, \quad (A-21)$$

and that the camera noise and RGB signals are uncorrelated,

$$E[n(R_i) R_i] = E[n(R_i) G_i] = E[n(R_i) B_i] = 0 \quad (A-22)$$

$$E[n(G_i) R_i] = E[n(G_i) G_i] = E[n(G_i) B_i] = 0, \quad (A-23)$$

Equation (A-16) can be written as

$$\Sigma_{RG} = E \left[\int i(\lambda_1) f_R(\lambda_1) [s_i(\lambda_1) - \bar{s}(\lambda_1)] d\lambda_1 \int i(\lambda_2) f_G(\lambda_2) [s_i(\lambda_2) - \bar{s}(\lambda_2)] d\lambda_2 \right]. \quad (A-24)$$

This equation describes covariance between the R and G pixel values. Similar equations can be derived for Σ_{RB} , for the R and B pixel values, and for Σ_{GB} , for the G and B pixel values.

References

- [1.] R. Chin, "Automated visual inspection: 1981 to 1987", *Computer Vision, Graphics, and Image Proc.*, 41:346-381, 1989.
- [2.] G. Healey, R. Kondepudy, "Radiometric CCD Camera Calibration and Noise Estimation", IEEE Conference on Computer Vision and Pattern Recognition, Champaign, Illinois, June 1992, pp. 90-95.
- [3.] B.K.P. Horn, *Robot Vision*, McGraw-Hill, New York, 1986.
- [4.] A.K. Jain, R.C. Dubes, *Algorithms for Clustering Data*, Prentice Hall, 1988.
- [5.] *Kodak Filters for Scientific and Technical Uses*, Eastman Kodak Company, Third Edition, 1981.
- [6.] C.S. McCamy, H. Marcus, J.G. Davidson, "A Color-Rendition Chart", *Journal of Applied Photographic Engineering*, Vol. 2, No. 3, Summer 1976, pp. 95-99.

[7.] M. Vriesenga, Colored Illumination for Maximizing Contrast in Machine Vision, Masters Thesis, Department of Electrical and Computer Engineering, University of California at Irvine, Irvine California, 92717, U.S.A, 1990.

[8.] M. Vriesenga, G. Healey, J. Sklansky, "Colored Illumination for enhancing the discriminability of objects in RGB images", Technical Report TP-91-3, Pattern Recognition and Image Modelling Project, Department of Electrical and Computer Engineering, University of California at Irvine, Irvine California, 92717, U.S.A, November 1991.

[9.] M. Vriesenga, G. Healey, J. Sklansky, "Colored illumination for enhancing discriminability in machine vision", Technical Report TP-92-4, Pattern Recognition and Image Modelling Project, Department of Electrical and Computer Engineering, University of California at Irvine, Irvine California, 92717, U.S.A, December 1992.

[10.] M. Vriesenga, G. Healey, K. Peleg, F. Hoffman, J. Sklansky, "Colored Illumination for Enhancing the Discriminability of Objects," Proceedings of the 8th Israeli Symposium on Artificial Intelligence and Computer Vision, December 1991.

Chapter 6 CLASSIFICATION PLANTLET SEGMENTS BY COLOR MACHINE VISION

Introduction

Traditional classification by machine vision is mainly based on black and white cameras, using grey scale level differences and geometrical features of the analyzed image as presented in chapter 4, ^{10,11}. In many cases the machine vision task can be considerably simplified by using color information in the inspected image, while the geometry is immaterial. We demonstrate this approach by showing that color information may be used for classifying plantlet segments as viable propagules or contaminants, by two types of color features, namely, the average intensity of the three basic color components and the number of pixels within a predefined region of the color space.

Materials and Methods

The potato plantlets were subcultured in transparent plastic boxes with dimensions 6cmx6cmx9cm. In each container, 10 plantlets were subcultured, arranged in two rows of three and one row of four plantlets. The containers remained in a growth chamber at constant temperature (24°C) and constant 24 h/d, artificial daylight illumination, for 24 d. They were then taken out of the container and randomly chopped into 1 cm length and roughly uniformly sized segments. Fig. 1 shows typical images of precut potato plantlet segments, some of which are viable propagules while others are just contaminants i.e. there is no propagation organ for generating a new plantlet. In a viable propagule, one or more leaves are usually attached to a stem segment. The propagation organs from which new plantlets can grow are tiny buds located at the connections between the leaves and the stem.

The segments were then inspected and classified by a color machine vision system, as described in the following paragraphs.

Each plantlet segment is placed in an illumination chamber on a backlit platform, which enhances the contrast between the plantlet and the background. The segment is illuminated from all directions by white fluorescent light, effectively eliminating shadows and specular reflections from the scene.

A video camera (Panasonic CCD camera model WV-D5000, NTSC standard) provides a composite video signal, which transmits the plantlet's image to a high quality video cassette recorder (VCR), where the image of the plantlet segment is recorded for future reproduction. The plantlet's image is also simultaneously transmitted to a color imaging board, where the image is digitized and some characteristic features of the image are computed. The image processing board, (model MVP-VME made by MATROX Corp.) is a VME-bus based board incorporating a Motorola 68000 central processing unit (CPU). The composite video signal from the camera, is connected to one of four available input channels and decomposed into the three main color components: red, green and blue (RGB). Each color component is stored in a 512x512 pixels frame buffer, with eight bit color resolution. The image can be processed and displayed as a unit or each frame buffer

Figure 1 Typical images of pre-cut potato plantlet segments.

individually. Some basic image processing functions are implemented in hardware (e.g. logical AND, OR, XOR operations) and performed also in real time. Additional features of the image processing board are edge detection functions, convolution, input and output functions and many graphic capabilities that make it easy to use.

The host computer comprises a Motorola VME-bus crate with a MVME-121 central processing board incorporating a Motorola 68010 CPU, which controls the image processing board and uses the characteristic features computed by it for classifying the plantlet segment as a viable propagule or as a contaminant.

The image processing board and the central processing board interface through a shared memory block where both, host MVME-121 CPU and MVP-VME local CPU, can read and write information that has to be passed from one to another (e.g. commands to the image processing board, results of image processing etc.). This facilitates very fast image processing as most of the computations and data transfers are effectively implemented in hardware. All the image processing algorithms were written in the "C" programming language and executed under the UNIX operating system.

Image Analysis and Classification

If a plantlet segment contains at least one leaf connected to a piece of stem, it is a good propagule. The color of the leaves in potato plantlets is dark green while the color of the stems is light yellow. By adding 0.25 ppm Ancyridol to the nutrient it is possible to increase this color difference significantly. As the colors are independent of the geometry, the classification task reduces to verifying that the image of the plantlet segment contains a sufficiently large dark green blob connected to a light yellow blob.

In this section it is shown how this color difference may be used for classifying plantlet segments as viable propagules or contaminants, without using geometric features, which are more computationally expensive. To this end, two types of color features are used, namely, the average intensity of the three basic color components and the number of pixels within a predefined region of the color space.

The image analysis and classification process is divided into three main stages as follows.

- (1) **Segmentation stage.** The image is segregated into distinct color regions, e.g. the background is separated from the plantlet image and the dark green regions representing leaves are separated from the light yellow regions representing stems.
- (2) **Feature extraction stage.** Some color features of the segmented regions are computed.
- (3) **Classification stage.** The plantlet segment is classified as a viable propagule or as a contaminant, by using the color features computed in stage two.

In the rest of this section, each of these three stages is described in detail.

Segmentation

The goal of the segmentation stage is to separate the image into distinct color regions.

Two basic operations are performed in this stage as follows.

- (1) Separation of the plantlet image from the background.
- (2) Identification of all pixels with color coordinates in a predefined region of the color space. This step is performed twice: once to identify all the dark green pixels (which are assumed to be leaf pixels) and once to identify all the light yellow pixels (which are assumed to be stem pixels).

It was found that the segmentation task could be greatly simplified by optimizing the illumination of the scene. This was accomplished by experimentally adjusting the intensity of the foreground and background illumination, whence it was possible to obtain sharp silhouettes of the plantlet segments while preserving (or even enhancing) the natural color difference between the stems and the leaves.

The separation of the plantlet from the background is based on intensity differences in the blue component of the color spectrum (blue wavelength). The main color components of the plantlets are in the green wavelength band, so the plantlet segment absorbs most of the blue color spectrum. On the other hand, the backlit white background contains almost all the visible spectrum including blue. Thus, the intensity of the background in the blue component is considerably greater than the intensity of the plantlet segment. This difference enhances the contrast between the plantlet segment and the background, hence the blue color component is optimal for the separation of the plantlet segment from the background. Fig. 2a shows a histogram of the blue color component, of a typical plantlet segment image and the segmentation threshold B_1 which may be used to separate the background from the plantlet image.

The identification of all dark green and light yellow pixels is based on intensity differences in the blue and the green components of the color spectrum. Fig. 2b shows a histogram of the green component of the color spectrum, of a typical plantlet segment image. It may be seen that the green component alone cannot be used for the segmentation task since the background intensity level therein is sometimes similar to that of the plantlet image (mainly the stem). However, a combination of the green and blue components can perform the segmentation task satisfactorily.

The red component does not provide additional information that can be used for the separation between the leaves and stems. This may be seen from the histogram in Fig. 2c, whence the peaks of the leaves and the stems are bunched together. It may be possible to separate the background from the plantlet image by a threshold such as R_1 but this is better accomplished by the green buffer histogram (Fig. 2b), where the distance between the background peak and the peak of the leaves with stems is significantly larger. For this reason, the red color component is not used in the segmentation stage.

The basic task of the segmentation algorithm is to define the regions in the color space that represent background, leaves or stems and then check, pixel by pixel, through all the pixels of the image, if each pixel belongs to the predefined color. In the most general case, the color of an object can be defined as a point in the RGB space, or as a set of points if the color is not uniform. In our case, the information of the red component is disregarded, so the segmentation process is performed in the green/blue plane. The principle of the segmentation algorithm is as follows.

Every pixel (i, j) that satisfies the inequalities:

$$G_2 < G_{ij} < G_1 \quad \text{and} \quad B_{ij} < B_1 \quad (1)$$

Figure 2 Typical histogram of the (a) blue, (b) green and (c) red component of the colour of a plantlet segment image using optimal front and back-lighting.

is a light yellow pixel, and every pixel that satisfies the inequalities:

$$G_3 < G_{ij} < G_2 \quad \text{and} \quad B_{ij} < B_1 \quad (2)$$

is a dark green pixel, where G_{ij} and B_{ij} are the intensity levels of the green and blue color component respectively, of pixel (i,j) . The threshold values B_1 , G_1 , G_2 and G_3 are computed from the respective histograms of the blue color (Fig. 2a) and the green color (Fig. 2b). Fig. 3 shows regions L and S in the green/blue plane, which define the dark green and light yellow regions respectively, while the remaining parts of the image are assumed to belong to the background.

The implementation of the segmentation algorithm minimizes the execution time by taking advantage of the built-in, hardware implemented functions of the image processing board.

Three types of basic operations are used by the segmentation algorithm. First, the contents of a frame buffer are altered by passing it through a look up table. Each such operation implements one of the inequalities (1) or (2). Secondly, a logical AND operation between two frame buffers is performed. This operation ensures that both inequalities (1) and (2) are satisfied, by the pixels that are matched to the predefined color, by the segmentation algorithm. Thirdly, a histogram of a frame buffer is generated for the computation of the area of the segmented image. These three basic operations are implemented in hardware and thus each one of them is performed in real time (one frame time per operation (FT) = 1/30 s). This high speed implementation of the color segmentation algorithm is essential for high speed classification of the plantlet segments, as required for cost effective practical application. Fig. 4 shows an example of the resulting binary images obtained by this algorithm, e.g. the dark green (leaf) and light yellow (stem) regions, separated from the whole plantlet segment image.

Feature extraction

Once the plantlet segment image is separated from the background, and the regions with the predefined colors are separated from the rest of the image, a feature extraction algorithm computes the characteristic features, that will be used in the classification stage.

Five color features of each plantlet segment were computed. The first three comprise the principal components of its color, i.e. average intensity level of the red (R_{av}), green (G_{av}) and blue (B_{av}) color components. The average color of segments that consist of leaf only will be very dark, while the average color of segments that consist of stem only will be very light. Segments that include both leaf and stem will have intermediate values of average color intensities. The average color of the segment represents a weighted ratio of the stem and leaf area. The two additional features are, the area of the dark green region (A_L) and area of the light yellow region (A_S). These features supply additional information about the absolute dimensions of the plantlet segment.

Four, two dimensional projections are used to study the five dimensional feature space, of a manually labelled set of plantlet segments, as depicted in Fig. 5 and 6. The average color intensity levels for each plantlet segment were computed and plotted in the red-green, red-blue and green-blue projection planes, as shown in Fig. 5. Viable propagules (L+S), are marked by asterisks, denoting segments wherein at least one leaf is connected to a stem. The contaminants, i.e. mainly separate leaves (L) or stems (S), are marked by circles and plus signs respectively.

It can be seen, that the viable propagules form a cluster approximately in the centre of the feature space, as shown by the projections on the red/green plane (Fig. 5a), blue/green plane (Fig. 5b) and blue/red plane (Fig. 5c). The separated leaves and bare stems tend to cluster near the two opposite sides of the central cluster. Furthermore, from the red/green projection plane of the feature space (Fig. 5a), it can be seen that there is a linear relation between the average intensity

Fig. 3 Regions of dark green (L) and light yellow (S) colour in the green/blue plane.

Figure 4 Binary image after segmentation. (a) Plantlet segment separated from the background, (b) Leaves and (c) Stems.

Legend

- * Good Leaf and stem
- + Bad Stem only
- o Bad Leaf only

Figure 5 Projection of the average color of pre-cut plantlet segments on the green/red (a), green/blue (b) and red/blue (c) plane.

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level of the red and green components. This relation indicates that the red and green components of the light spectrum are absorbed and reflected from the plantlets in a similar manner. Thus, the intensity levels of the green and red color components behave similarly. From the green/blue and red/blue projection planes of the feature space (Fig. 5b and Fig. 5c respectively), it can be seen that there is no significant difference between the average intensity level of the blue component of viable propagules and contaminants, since the blue spectrum energy is almost equally absorbed by all the parts of the plantlet segment.

Fig. 6 shows another two dimensional projection of the same feature space, wherein the area of the dark green region is plotted versus the area of the light yellow region. Here too, the viable propagules form a cluster at the centre of the projection plane, while the contaminants tend to form two rather elongated clusters along the two axis.

The values of the means, μ , and standard deviations, σ , of the above five features, were computed for a manually labelled training set of 1023 plantlet segments.

The results are summarized in Table 1. Note the significant differences between the means μ_w , μ_L , μ_S and standard deviations σ_w , σ_L , σ_S of the good and bad propagules, in the five characterizing features. The larger these differences are, the better will be the classifier.

Classification

The images of a test set of pre-cut plantlet segments were classified as GOOD (viable propagule), or BAD (contaminant) by three different classification algorithms, "classifiers". A good classifier should be accurate and suitable for high speed implementation. Unfortunately, classifiers do not usually possess both properties at once. Accurate classification is achieved by sophisticated algorithms which demand high computational resources. On the other hand, high speed classifiers are usually less accurate, so a balance must be struck between high speed classification and accuracy. In this study, three different classification algorithms were tested.

The expected performance of the three classifiers, in terms of training mode and classification speed, as well as accuracy, were estimated by simulated classification tests of pre-cut plantlet segments. The classification errors C_r , of each classifier were calculated as the number of misclassified segments, divided by the total number of segments that were classified:

$$C_r = N_e / N_t \quad (3)$$

where N_e total number of misclassified segments
 N_t total number of classified segments

1) Using the parameters of the five color features from Table 1, a simple classical maximal log-likelihood (MXL) classifier was used, e.g. Duda and Hart¹², assuming that the features are normally distributed and independent of each other.

By these assumptions, the probability of a good plantlet segment is given by:

$$P_w = - \sum_{i=1}^k \left[\text{Log}(\sigma_{wi}) + \frac{(F_i - \mu_{wi})^2}{2 \sigma_{wi}^2} \right] \quad (4)$$

The probability of bad plantlet segment is given by:

Table 1
Parameters of color Feature Distributions

Feature f_i	Good		Bad		Bad	
	(leaf+stem)		(leaves only)		(stems only)	
	μ_w	σ_w	μ_L	σ_L	μ_S	σ_S
1. Mean intensity of red color R_{av}	169.7	14.5	135.7	9.2	193.0	14.3
2. Mean intensity of green color G_{av}	186.9	15.9	145.6	10.6	215.4	13.9
3. Mean intensity of blue color B_{av}	142.0	10.6	130.8	4.4	146.3	12.1
4. Dark green area (leaves) A_L (mm ²)	7.3	8.8	15.4	8.4	0.6	4.8
5. Light yellow area (stems) A_S (mm ²)	13.5	6.3	1.7	4.4	10.6	5.5

$$P_L = -\sum_{i=1}^k \left[\text{Log}(\sigma_{Li}) + \frac{(F_i - \mu_{Li})^2}{2 \sigma_{Li}^2} \right] \quad (5)$$

or

$$P_S = -\sum_{i=1}^k \left[\text{Log}(\sigma_{Si}) + \frac{(F_i - \mu_{Si})^2}{2 \sigma_{Si}^2} \right] \quad (6)$$

Here F_i is the value of the i -th feature in the test set, k is the number of features, while μ_W , σ_W , μ_L , σ_L , μ_S and σ_S are the mean and standard deviation of the i -th feature from Table 1. The pre-cut plantlet segment is classified to the class with the maximal probability. This classifier does not require any training. Using the average values of the features among the classes and their standard deviations, the classification process is simply based on computing P_W , P_L and P_S by eqs. (4), (5) and (6). As the number of numerical operations is relatively small, a maximal log-likelihood classifier is suitable for high speed classification, but its accuracy is quite poor if the actual class conditional probability densities of the measured features are not normal.

2) Considerably better performance may be obtained by a K-nearest neighbors (KNN) classifier, e.g. Duda and Hart¹². As one would expect from the name, the KNN rule classifies a feature vector of a given plantlet segment, by assigning it the label most frequently represented among the K nearest samples, in the training set. In other words, a decision is made by examining the labels on the K nearest neighbors and taking a vote. To avoid ties K should be odd, hence we used $K=3$. The training procedure of this classifier is quite simple. It consists of just storing in computer memory, all the feature vectors of the labelled training set. However, the main disadvantage is slow speed and large memory requirement, since the entire training set must be stored at run time. The classification process is cumbersome, because each classification decision is preceded by a large number of computations, to determine the distances between all the feature vectors in the training set and the feature vector of the plantlet segment that has to be classified.

3) Neural Networks based classifiers have the potential for relatively good accuracy and very high speed execution, due to their inherent parallel computation structure, which can be implemented by dedicated hardware. On the other hand their training procedure can be long and tedious.

In this study a Boltzmann Perceptron Network (BPN), due to Yair and Gersho¹³ was used. The topology of this BPN is depicted in Fig. 7, wherein 5 inputs, 3 hidden nodes, and 3 output nodes are connected as shown. In the training stage, exemplars of the five measured features from the training set were clipped to the respective input nodes. A value of 1, denoted the correct class label at the appropriate output node, while 0 values were clipped to the other two output nodes. A special training algorithm, due to Yair and Gersho¹³ was used to train this BPN. This algorithm incorporates a partial conjugate gradient minimization scheme, similarly to the classical back-propagation algorithm, that is used in regular perceptron networks. Although the training stage of the network is tedious, the classification process at run time is very fast. Given the values of five measured features of a plantlet segment, the BPN automatically estimates the class probabilities P_W , P_L and P_S , while $(P_W + P_L + P_S = 1)$. The plantlet segment is then classified to the class with the maximal probability.

Inputs

Red intensity
Green intensity
Blue intensity
Dark green area
Light yellow area

Outputs

Leaf only
Leaf + stem
Stem only

Hidden nodes

Figure 7 Topology of Boltzmann-Perceptron Neural network

The results of the simulated classification tests, by the above three classifiers are summarized in Table 2. It may be seen that the misclassification rate of the KNN classifier was lowest (7.3%), while that of the maximum log-likelihood classifier (MXL), was highest (12.7%). However, the MXL classifier performed better in terms of misclassification of the bad segments as good: 9.3% misclassified by MXL versus 31% and 32.1%, by the KNN and BPN classifiers respectively.

The total misclassification rate of the BPN classifier was inbetween those of the MXL and KNN classifiers, i.e. 9.9%. Given the high speed classification requirements of this task, it seems like the BPN is a good compromise between the MXL and KNN classifiers.

Combination of geometric and color features

Classification of plantlet segments by geometric features only yields a classification error of 12%. By using color features only the classification error was 7%, which is still too high for practical implementation. In order to decrease the classification errors further, color features were combined with geometric features.

The improvement in classification accuracy by combining color and geometric features was evaluated by six different classifiers:

- 1) K-nearest neighbors classification algorithm with K=3 (NN3)
- 2) K-nearest neighbors classification algorithm with K=5 (NN5)
- 3) Classifier based on a Boltzman Perceptron Network with 3 hidden layers (BPN3)
- 4) Classifier based on a Boltzman Perceptron Network with 5 hidden layers (BPN5)
- 5) Classifier based on a Boltzman Perceptron Network with 6 hidden layers (BPN6)
- 6) Maximal Log-Likelihood classifier (MXL)

Figure 8 illustrates the classification errors of the six different classifiers. All classifiers were trained with the same training set and tested with the same test set. It can be seen that independently of the classification algorithm, classification with color features only is more accurate than classification with geometric features only. In addition, combination of color and geometric features reduces even more the misclassification rates. According to Figure 1, by combining color and geometric features the classification error was reduced to 5% while using the best classification algorithm, which in this case was NN3.

Conclusions

Using color machine vision and a Neural Network based classifier, we have laid a basis for a practical system, which may be used for automatic classification of tissue culture segments of potato plantlets. Instead of the conventional use of black and white cameras and geometric features, we use color features only, and take advantage of the hardware implemented color frame manipulation capabilities, which are now available in most commercial imaging boards. This facilitates high speed plantlet classification by automatic subculturing systems.

Although the simulated classification results are encouraging there is still considerable room for improvement before such a system can be used in practice. Particularly, we have to reduce the

rather high rate of missclassifying good plantlet segments as bad.

In order to improve the performance of the system color and geometric features were combined together. The above results indicate that the presence or the absence of axillary buds in the plantlet segment, is described better by color features only than by geometric features only. This can be seen by the lower misclassification rate obtained by classification using color features only rather than geometrical features. The further reduction of the misclassification rate by combining color and geometric features indicate that they describe the presence of axillary buds in a different way. There are cases where geometric features fail to correctly identify plantlet segments that contain axillary buds, while color features succeed. The opposite might occur as well. Consequently, combining color and geometric features exploits the advantages of both types of features and reduce the classification errors.

Although combining of color and geometric features reduces the misclassification to 5%, this is still not enough for practical implementation. Thus, a more efficient classification algorithm is required to render the misclassification rate to negligible levels. To this end a classifier voting algorithm was developed as described in the next chapter.

Table 2
Results of Classification Tests by color Features

		Label assigned by classifier		
		Good (leaf+stem)	Bad (leaves only)	Bad (stems only)
Plantlet segment label	KNN	95.7%	1.2%	3.1%
	MXL	83.6%	4.1%	12.3%
	BPN	92.6%	2.8%	4.6%
Good	KNN	3.9%	96.1%	0.0%
	MXL	5.0%	95.0%	0.0%
	BPN	5.0%	95.0%	0.0%
Bad (leaves only)	KNN	27.1%	0.0%	72.9%
	MXL	4.3%	0.0%	95.7%
	BPN	27.1%	1.4%	71.4%
Bad (stems only)	KNN	7.3%		
	MXL	12.7%		
	BPN	9.9%		
Total misclassification	KNN			
	MXL			
	BPN

■ **Figure 8** : Classification error of potato plantlet segments, using color and geometric features.

Chapter 7 MINIMAL MISCLASSIFICATION BY VOTING CLASSIFIERS

In this chapter a new algorithm is presented, whereby the decisions of several classifiers are combined. The goal of this algorithm is to classify objects with minimum misclassification. In what follows, the decision process is presented first in general terms, and then the algorithm is applied for classifying potato shoots from tissue cultures.

This classification algorithm combines the decision of several classifiers by a voting process. The decision process of classifier voting is illustrated in figure 1. At the beginning, each classifier classifies the object to one of the classes. Then, the individual decisions are combined by taking a vote. In this project, two ways of combining the votes of the individual classifiers were studied:

- 1) Classifier voting by consensus
- 2) Classifier voting by majority

Classifier voting by consensus

The decision process of classifier voting by consensus is depicted in figure 2. The object is classified to class i if all individual classifiers agree that the object belongs to that class. Otherwise, the object is classified to a residual class. The residual class has to be treated separately. Some possible treatments of the residual class are presented later.

Using the six classifiers presented in Chapter 6, the plantlet segments were classified by consensus voting of two and three classifiers. Tables 1 and 2 show the results of classification by consensus voting with two and three classifiers respectively. The leftmost column shows the names of the classifiers which participate in the voting process.

Columns Cr1, Cr2 and Cr3 depict the contamination index when the plantlet segments are classified by the first, the second and the third classifier respectively. Column Crcon represents the contamination index of classification by consensus voting. Figures 3 and 4 compare graphically the contamination indices obtained by individual classifiers and their combination by consensus voting. It can be seen that the combination of classifiers increases the classification accuracy. For example, when MXL is combined with NN3, the contamination index decreases by 2.8% compared to NN3 alone, and by 8.1% compared to MXL alone.

The lowest contamination index is obtained when NN3, BP3 and MXL are combined. In this case the contamination index is 1.37%, which is practically very close to zero.

Column Pu in tables 1 and 2 depicts the residual class, expressed as a percentage of the whole test set. It can be seen that the residual class varies from 4% to 15% of the inspected plantlet set.

Figure 1: The decision process of classifier voting

Figure 2: The decision process of classifier voting by consensus

Classifiers	Cr1	Cr2	Crcn	Pu	Pgl	Crc+b	J1	J2	Jc	Jc+b	Jcs	Crcs	Pgl's
	[%]	[%]	[%]	[%]	[%]	[%]	cost	cost	cost	cost	cost	[%]	[%]
	1	2	3	4	5	6	7	8	9	10	11	12	13
BP5 BP6	6.74	7.53	4.93	4.89	2.56	6.74	40.3	43.5	32.5	40.3	12.3	42	1
BP3 BP6	6.26	7.53	4.34	5.47	3.56	6.26	34.5	43.5	30.8	34.5	10	39.29	1.85
BP3 BP5	6.26	6.74	4.68	4.01	3.13	6.26	34.5	40.3	33	34.5	7	43.9	1.85
BP6 MXL	7.53	10.4	2.91	12.8	11.7	7.53	43.5	32	30	43.5	34	38.93	1
BP5 MXL	6.74	10.4	2.47	12.8	12	6.74	40.3	32	29	40.3	32.3	35.88	0.85
BP3 MXL	6.26	10.4	2.77	11.7	11.7	6.26	34.5	32	29.3	34.5	25.8	32.5	1
BP6 NN3	7.53	5.08	3.14	6.74	3.56	5.08	43.5	29.3	23.8	29.3	11.8	31.88	1.14
BP6 NN5	7.53	7.62	3.83	8.11	4.69	7.53	43.5	41.8	28.8	43.5	23	49.4	1.99
BP5 NN5	6.74	7.62	3.99	6.94	4.98	6.74	40.3	41.8	32.3	40.3	16.8	43.66	1.85
BP5 NN3	6.74	5.08	3.31	5.57	2.99	5.08	40.3	29.3	24.3	29.3	10.3	35.09	1.14
BP3 NN3	6.26	5.08	2.71	6.26	3.56	5.08	34.5	29.3	20.8	29.3	14.8	40.63	1.14
BP3 NN5	6.26	7.62	3.78	6.84	4.98	6.26	34.5	41.8	29.8	34.5	13.5	40	2.13
NN5 MXL	7.62	10.4	4.25	10.4	8.96	7.62	41.8	32	29	41.8	28.5	36.79	0
NN3 MXL	5.08	10.4	2.32	11.3	11.2	5.08	29.3	32	27	29.3	22	26.72	0.14
NN3 NN5	5.08	7.62	3.34	6.45	3.98	5.08	29.3	41.8	24.8	29.3	11.5	30.3	1

Table 1: Classification results of potato plantlet segments by consensus voting of two classifiers

Classifiers	Ct1	Ct2	Ct3	Crcon	Pu	Pgl	Crc+b	J1	J2	J3	Jc	Jc+b	Jcs	Crcs	PglS
	[%]	[%]	[%]	[%]	[%]	[%]	[%]	cost	cost	cost	cost	cost	cost	[%]	[%]
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
BP3 BP5 BP6	6.26	6.74	7.53	3.68	7.14	4.55	6.26	34.5	40.3	43.5	29.3	34.5	13.3	39.7	2.42
MXL BP5 BP6	10.4	6.74	7.53	2.08	15.3	13.1	6.74	32	40.3	43.5	30	40.3	33.3	32.7	1.42
MXL BP3 BP5	10.4	6.26	6.74	1.94	14.3	13.4	6.26	32	34.5	40.3	29.8	34.5	28.3	32.2	1.99
MXL BP3 BP6	10.4	6.26	7.53	1.95	15	13.4	6.26	32	34.5	43.5	29.8	34.5	28.3	30.7	1.99
NN5 BP5 BP6	7.62	6.74	7.53	3.15	9.97	6.12	6.74	41.8	40.3	43.5	28	40.3	23	39.2	1.99
NN5 BP3 BP6	7.62	6.26	7.53	2.94	10.2	6.54	6.26	41.8	34.5	43.5	27.8	34.5	18.3	35.6	2.7
NN5 BP3 BP5	7.62	6.26	6.74	3.22	8.9	6.54	6.26	41.8	34.5	40.3	30.5	34.5	15.5	37.4	2.84
NN5 MXL BP3	7.62	10.4	6.26	1.83	14.5	12.8	6.26	41.8	32	34.5	28.5	34.5	28.5	32.4	2.13
NN5 MXL BP5	7.62	10.4	6.74	1.73	15.1	12.9	6.74	41.8	32	40.3	29	40.3	34	35.1	1.85
NN5 MXL BP6	7.62	10.4	7.53	2.09	15.6	12.7	7.53	41.8	32	43.5	29.3	43.5	36.5	36.9	1.99
NN3 BP5 BP6	5.08	6.74	7.53	2.78	8.6	4.55	5.08	29.3	40.3	43.5	23	29.3	14.3	29.6	1.28
NN3 BP3 BP6	5.08	6.26	7.53	2.15	9.19	5.26	5.08	29.3	34.5	43.5	20.8	29.3	17.8	34	1.56
NN3 BP3 BP5	5.08	6.26	6.74	2.44	7.92	4.84	5.08	29.3	34.5	40.3	21.8	29.3	16	35.8	1.42
NN3 NN5 BP6	5.08	7.62	7.53	2.19	10.7	6.12	5.08	29.3	41.8	43.5	22.3	29.3	17.8	29.4	1.56
NN3 NN5 BP5	5.08	7.62	6.74	2.48	9.48	5.97	5.08	29.3	41.8	40.3	23.8	29.3	16	29.9	1.42
NN3 NN5 BP3	5.08	7.62	6.26	2.06	9.78	6.26	5.08	29.3	41.8	34.5	21.3	29.3	19	33	1.42
NN3 MXL BP6	5.08	10.4	7.53	1.5	15.4	13.2	5.08	29.3	32	43.5	28	29.3	24.5	24.7	1.14
NN3 MXL BP5	5.08	10.4	6.74	1.49	14.9	13.1	5.08	29.3	32	40.3	27.8	29.3	24.5	25.7	1.14
NN3 MXL BP3	5.08	10.4	6.26	1.37	14.7	13.2	5.08	29.3	32	34.5	27.3	29.3	25.3	26.7	1.14
NN3 MXL NN5	5.08	10.4	7.62	1.71	14.1	12.1	5.08	29.3	32	41.8	27	29.3	23.5	25.7	1

Table 2: Results of potato segments classification by three classifier consensus voting

■ **Figure 3** Classification errors and percent of unclassified plant segments, in classification experiments by consensus voting of two classifiers.

■ **Figure 4** Classification errors in three classifier consensus voting experiments.

The rest of the columns of tables 1 and 2 refer to specific treatments of the residual class and will be explained in the next paragraphs.

The contamination index does not take into account the residual class, so it cannot be used to compare between different policies for its disposition. To do so, a cost function was adopted, for expressing the loss of potentially good plantlets due to misclassification caused by a specific policy. The cost function, J , is defined as follows:

$$J = \sum_{i=1}^k \sum_{\substack{j=1 \\ i \neq j}}^k C_{ij} N_{ij}$$

where

J – cost function

K – number of classes

C_{ij} – cost of contaminating class j by segments that belong to class i

N_{ij} – number of segments that belong to class i but were classified to class j

When a good segment is thrown away, a potentially good plantlet is lost. On the other hand, if an unsuitable segment is transplanted, the whole box, with nine more segments, in which it is planted may be infected. Assuming that one third of the box will be damaged, and bearing in mind that the transplanted unsuitable segment takes the place of another potentially good plantlet, the average loss is about four potentially good plantlets. Based on the above loss estimation, the cost ratio of contaminating the good and the bad classes is 1:4.

Columns J1, J2 and J3 in tables 1 and 2 depict the cost function of the individual classifiers listed in the leftmost column respectively.

The residual class was treated in the following ways:

1) The residual class is rejected.

In this case, all good segments included in the residual class are lost. Column Pgl in tables 1 and 2 depicts the good segments lost when this policy is used, expressed as a percentage of the total number of the good segments in the test set. Column Jc in tables 1 and 2 depicts the cost function of this policy. It can be seen that the combination of classifiers that give the minimum contamination index is different from the combination that yields the minimum cost function. For example, when the segments are classified by consensus voting of three classifiers, the minimum contamination index is obtained by combining NN3, MXL and BP3. However, the minimum cost function is obtained by combining NN3, BP3 and BP6.

This is due to the large residual class of the first combination, i.e. 14.7%, compared to the moderate residual class (9.1%), and relatively low contamination index (2.15%), of the second combination.

2) The residual class is classified separately by the best classifier.

Column J_{c+b} in tables 1 and 2 depicts the cost function when the residual class is classified separately by the best classifier, among those who participate in the voting process. It can be seen that the value of J_{c+b} is identical to the value of the cost function when using the best classifier alone. Nevertheless, voting by consensus is a two stage classification process. In the first stage the contamination index is low, but a residual class is present. In the second stage, the residual class is separately classified.

As the residual class is composed of the more problematic plantlet segments, their classification is more difficult and consequently more erroneous. Columns J_{cs} , C_{cs} and P_{gls} in tables 1 and 2 show the cost function, contamination index and percentage of good segments lost of the residual class. As expected, the classification errors are much higher than in the plantlet segments that were classified by consensus voting.

3) The residual class is returned to the imaging system and reclassified by consensus voting.

Normally, each plantlet segment is classified by the characteristic features that were extracted from a single two-dimensional view of the plantlet segment, which is three dimensional. If the measured features do not enable classification by consensus, this policy gives the imaging system another chance, to obtain a better view of the plantlet segment. As there is a good chance that the plantlet segment will face the camera in a different way, the new set of characteristic features thus extracted, may enable to correctly classify the plantlet segment.

Fig 5 shows an example of the classification error and the size of the residual class as a function of the number of repeated classification trials. It can be seen that the repeated classification trials reduce the size of the residual class by 3%, while the contamination index increases only by 1%. Tables 3 and 4 show the classification results of repeated consensus voting with two and three classifiers respectively. Columns Cr_1 , Cr_2 and Cr_3 show the contamination index of the individual classifiers that participate in the voting process. Columns Cr_{con1} and Cr_{con20} show the contamination index after the first and the twentieth classification trial respectively. Columns P_{gl1} and P_{gl20} show the percentage of good segments in the residual class after the first and after the twentieth classification trial respectively. It can be seen that in all cases, repeated classification trials decrease the number of good segments in the residual class and increase the contamination index. Nevertheless, the enlargement of the contamination index is less significant than the corresponding reduction of the good segments included in the residual class and thus the overall classification performance is improved.

Figure 5: Classification error and size of residual class, as a function of the number of repeated classification trials.

Classifier	Cr1	Cr2	Crcon1	Pgl1	Crcon20	Pgl20
	[%]	[%]	[%]	[%]	[%]	[%]
	1	2	3	4	5	6
BP5 BP6	8.78	9.91	6.5	3.05	6.93	0.9
BP3 BP6	7.77	9.83	5.84	3.65	6.21	1.19
BP3 BP5	7.89	8.94	6.25	3.55	6.42	1.19
BP6 MXL	9.79	10.14	2.66	13.93	3.44	8.73
BP5 MXL	8.9	10.12	2.57	13.53	2.8	9.62
BP3 MXL	7.69	10.26	3.11	12.74	3.72	7.49
BP6 NN3	10.06	6.68	3.8	4.68	3.97	1.79
BP6 NN5	10.06	9.4	4.6	5.8	4.83	3.28
BP5 NN5	8.82	9.56	5.2	5.7	5.67	2.99
BP5 NN3	8.8	6.45	4.24	3.09	4.86	1.19
BP3 NN3	7.83	6.76	3.45	3.91	3.84	1.79
BP3 NN5	7.65	9.19	4.47	5.84	5.46	2.39
NN5 MXL	9.42	10.18	4	10.85	4.9	7.19
NN3 MXL	6.76	10.35	2.49	13.43	3.02	9.28
NN3 NN5	6.51	9.52	4.24	4.54	4.51	2.09

Table 3: Classification results of potato plantlet segments by repeated consensus voting by two classifiers

Classifier	Cr1	Cr2	Cr3	Crcon1	Pgl1	Crcon20	Pgl20
	[%]	[%]	[%]	[%]	[%]	[%]	[%]
	1	2	3	4	5	6	7
BP3 BP5 BP6	7.78	8.83	9.83	5.08	4.69	5.43	1.79
MXL BP5 BP6	10.19	8.81	9.79	2.03	14.96	2.3	10.45
MXL BP3 BP5	10.11	7.62	8.88	2.16	14.78	2.4	9.85
MXL BP3 BP6	10.26	7.71	9.69	2.15	14.87	2.45	9.94
NN5 BP5 BP6	9.29	8.9	9.83	3.72	7.28	4.37	3.88
NN5 BP3 BP6	9.3	7.8	10.12	3.85	7.58	4.2	4.18
NN5 BP3 BP5	9.48	7.57	8.83	4.15	7.25	4.6	4.18
NN5 MXL BP3	9.41	10.11	7.71	1.78	14.36	2.44	9.58
NN5 MXL BP5	9.53	10	8.78	1.56	14.75	2.06	10.78
NN5 MXL BP6	9.69	10.21	9.97	2	14.72	2.57	10.24
NN3 BP5 BP6	6.68	8.87	9.88	3.45	5.31	3.92	2.09
NN3 BP3 BP6	6.56	7.82	9.97	2.46	6.06	2.93	2.69
NN3 BP3 BP5	6.59	7.68	8.9	3.06	5.43	3.65	2.39
NN3 NN5 BP6	6.63	9.34	9.91	2.43	6.69	2.54	4.48
NN3 NN5 BP5	6.73	9.32	8.9	3.03	6.72	3.37	3.88
NN3 NN5 BP3	6.57	9.34	7.9	2.59	6.72	2.89	3.88
NN3 MXL BP6	6.78	10.54	9.93	1.49	15.88	1.91	11.13
NN3 MXL BP5	6.5	10.3	8.85	1.45	15.34	1.86	11.1
NN3 MXL BP3	6.64	10.33	7.64	1.49	15.22	1.85	10.48
NN3 MXL NN5	6.54	10.12	9.39	1.68	14.27	2.28	10.51

Table 4: Classification results of potato plantlet segments by repeated consensus voting by three classifiers

Classifiers	Cr1	Cr2	Cr3	Crmaj	Pu	Pgl	Crm+b	J1	J2	J3	Jm	Jm+b	Jms	Crcs	Pgls
	[%]	[%]	[%]	[%]	[%]	[%]	[%]	cost	cost	cost	cost	cost	cost	[%]	[%]
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
BP3 BP5 BP6	6.26	6.74	7.53	6.46	0.1	0.14	6.55	34.5	40.3	43.5	37.8	37.8	0.3	100	0.14
MXL BP5 BP6	10.4	6.74	7.53	5.87	0	0	5.87	32	40.3	43.5	31.5	31.5	0	0	0
MXL BP3 BP5	10.4	6.26	6.74	5.77	0	0	5.77	32	34.5	40.3	31.8	31.8	0	0	0
MXL BP3 BP6	10.4	6.26	7.53	5.77	0.1	0.14	5.87	32	34.5	43.5	30.5	30.5	0.3	100	0.14
NN5 BP5 BP6	7.62	6.74	7.53	6.26	0	0	6.26	41.8	40.3	43.5	37.5	37.5	0	0	0
NN5 BP3 BP6	7.62	6.26	7.53	5.87	0.1	0.14	5.96	41.8	34.5	43.5	33.8	33.8	0.3	100	0.14
NN5 BP3 BP5	7.62	6.26	6.74	5.87	0	0	5.87	41.8	34.5	40.3	34	34	0	0	0
NN5 MXL BP3	7.62	10.4	6.26	6.65	0	0	6.65	41.8	32	34.5	31	31	0	0	0
NN5 MXL BP5	7.62	10.4	6.74	6.74	0	0	6.74	41.8	32	40.3	32.3	32.3	0	0	0
NN5 MXL BP6	7.62	10.4	7.53	6.35	0	0	6.35	41.8	32	43.5	29.3	29.3	0	0	0
NN3 BP5 BP6	5.08	6.74	7.53	5.67	0	0	5.67	29.3	40.3	43.5	34.5	34.5	0	0	0
NN3 BP3 BP6	5.08	6.26	7.53	5.68	0.1	0.14	5.67	29.3	34.5	43.5	33.8	33.5	0.3	100	0.14
NN3 BP3 BP5	5.08	6.26	6.74	5.67	0	0	5.67	29.3	34.5	40.3	34.5	34.5	0	0	0
NN3 NN5 BP6	5.08	7.62	7.53	5.67	0	0	5.67	29.3	41.8	43.5	32.8	32.8	0	0	0
NN3 NN5 BP5	5.08	7.62	6.74	5.47	0	0	5.47	29.3	41.8	40.3	33.8	33.8	0	0	0
NN3 NN5 BP3	5.08	7.62	6.26	5.47	0	0	5.47	29.3	41.8	34.5	32.8	32.8	0	0	0
NN3 MXL BP6	5.08	10.4	7.53	4.99	0	0	4.99	29.3	32	43.5	24.8	24.8	0	0	0
NN3 MXL BP5	5.08	10.4	6.74	4.79	0	0	4.79	29.3	32	40.3	24.8	24.8	0	0	0
NN3 MXL BP3	5.08	10.4	6.26	4.69	0	0	4.69	29.3	32	34.5	22.5	22.5	0	0	0
NN3 MXL NN5	5.08	10.4	7.62	6.06	0	0	6.06	29.3	32	41.8	26.8	26.8	0	0	0

Table 5: Classification results of potato plantlet segments by majority voting of three classifiers.

Classifier voting by majority

The majority voting criterion was tested, concurrently with the consensus voting policy. According to this policy, the plantlet segment is classified to the class that collects the largest number of votes. In case of tie, the segment is classified to a residual class. At least three individual classifiers are required, for classifier voting by majority.

Using the six classifiers presented in Chapter 6, the plantlet segments were classified by majority voting of various combinations of three classifiers. Table 5 shows the results of classification by majority voting. The leftmost column shows the names of the classifiers which participate in the voting process. The rest of the columns represent the same variables as in consensus voting. It can be seen that combination of classifiers by majority voting does not always increase the classification accuracy. For example, when NN3, BP3 and BP6 are combined together, the contamination index, Crmaj, increases by 0.6% compared to NN3 alone. In other cases, the contamination index decreases, as for example in the combination of NN3, MXL and BP3.

The lowest contamination index is obtained when NN3, BP3 and MXL are combined. In this case the contamination index is 4.7%, which is 0.4% lower than the contamination index of NN3 alone. Column Pu in table 5 depicts the residual class, expressed as a percentage of the whole test set. It can be seen that the residual class is practically always zero.

In summary, the proposed algorithm of classifier voting enables classification of potato shoot segments with negligible classification error of 1.37%. Such very low missclassification rates are essential for practical application of this system, in commercial automatic machines, for mass micropropagation.

Chapter 8 TASK FOCUSED MODELING OF POTATO PLANTLETS

INTRODUCTION

Machine vision systems analyze image data to carry out automation tasks. Our interest is in machine vision systems that rely on models to achieve their designed task. If a machine vision system is required to perform its task in real-time, then it must devote its resources to those portions of the data that contain information used for the completion of the task. Machine vision systems that do not conserve their resources may produce models that contain more information than is required to complete the task. This over-modeling is often unacceptable for machine vision systems that must perform their task in real-time. For example, in finding the cutting sites on a plant for clonal micropropagation, it is unnecessary to model the fine structure of the leaves, the root system, or the apex of the plant. Instead, it suffices to find and model the location of the regenerative buds along the plant stem. The cutting sites along the plant stem can then be deduced from this model.

In this part, we propose a modeling scheme for machine vision systems, called *task-focused modeling*, which expends a minimal effort in constructing a model having sufficient detail to advance the completion of the task. We illustrate task-focused modeling by an example involving real-time micropropagation of plants in automated agriculture.

In Section 2, we present an example of task-focused modeling for the real-time micropropagation of plants. In Section 3, we present a generalized scheme for task-focused modeling. Finally, in Section 4, we make some conclusions about the effectiveness of task-focused modeling for real-time automated agriculture.

TASK-FOCUSED MACHINE VISION FOR REAL-TIME MICROPROPAGATION OF PLANTS

The nature of the objects in the scene places constraints on the type of models and modeling algorithms that can be used in a task-focused machine vision system. When nonrigid objects, such as plants, are processed using machine vision, the modeling technique must adapt to variations in plant morphology, orientation, and scale. In addition, to represent the different aspects of each plant, different information is required at different locations in the model. Therefore, the model produced by a task-focused technique must adapt to regions of interest that vary from image to image.

We present an agricultural example of a task-focused machine vision system. This system is designed for automating the real-time micropropagation of plants. The task for this system is to mark the cutting sites on tissue culture plants in a manner approximating those sites found by humans. It carries out this task by constructing a model from which the cutting sites are discovered and marked. Our example consists of a series of iterations involving information extraction, model construction, and model analysis. The focusing of the modeling takes place within the "model analysis" process.

Figure 1 - A typical image of a plant that is processed using task-focused modeling.

Figure 1 illustrates a typical image of a plant that is processed by our machine vision system. The iterations required to process this image using our task-focused modeling technique are:

Iteration One:

Information Extraction and Model Construction: In the first iteration, the machine vision system separates the foreground from the background and establishes a single rectangular region of interest in the image data. This region of interest delineates part of the image data where more information about the plant can be extracted in the following iterations. Figure 2 illustrates this region of interest as a shaded rectangular region designated A1.

Model Analysis: None.

Iteration Two:

Information Extraction and Model Construction: In the second iteration, the machine vision system finds and marks buds (O), leaf-stem intersections (□), and the end point of the stem (X). Figure 3 illustrates the model that results from this information. Buds that are not detected

are called *false negatives* while buds that are detected but do not exist are called *false positives*. The model in Figure 3 contains three false negatives and two false positives.

Model Analysis: In this step, the machine vision system fits a straight line to the detected buds, leaf-stem intersections, and the end point of the stem. The distance between a candidate bud and the line varies inversely as the likelihood that the candidate is a true bud. Thus, the machine vision system recognizes the candidate bud in the lower right-hand corner of Figure 3(a) as a false positive and removes it from the model. Finally, the machine vision system identifies potential bud-stem pairs in the model and marks the associated regions of interest in the image data. Figure 3(b) illustrates these regions of interest as shaded rectangular regions designated B1 to B5.

Iteration Three:

Information Extraction and Model Construction: In the third iteration, the connection between bud-stem pairs is discovered and is represented in the model by joining and coloring the corresponding nodes. The connectivity is found by tracing along the plant stem in the image data.

Model Analysis: The connected bud-stem pairs are considered high confidence buds and therefore further modeling in regions B1 to B5 is unnecessary. In an ideal case, where there are no unmatched buds and/or leaf-stem intersections, the model would be complete. However, the model in Figure 4 does have unmatched buds and leaf stem intersections. Therefore, further modeling is required in the regions associated with these objects. Figure 4 illustrates the new regions of interest as shaded regions designated C1 to C4. At this point, there are two different types of modeling defects that must be resolved. In regions C1, C3, and C4 there are leaf-stem intersections for which there is no corresponding bud. This suggests that one or more false negatives might be present in the model. In region C2 there is a candidate bud for which there is no corresponding leaf. This suggests that of a false positive might be present in the model.

Iteration Four:

Information Extraction and Model Construction: In the fourth iteration, leaf blobs are extracted from regions C1, C3, and C4 and are added to the model. Additionally, these regions are thoroughly searched for new candidate buds that can be paired with the corresponding leaf. In Figure 5, the new candidate buds, which were previously false negatives, are detected in regions C1 and C4 and are added to the model. No new candidate bud is discovered in region C3. Finally, region C2 is searched more thoroughly for a leaf to which the candidate bud can be paired. In this region, no new leaf is found since candidate leaves must occur to the right of the bud on the plant stem.

Model Analysis: In this analysis step, the machine vision system decides that the new bud-leaf pairs discovered in regions C1 and C4 result in high confidence buds. Furthermore, the lack of an acceptable leaf in region C2 suggests that the bud is a false positive and is therefore deleted from the model. The only remaining model defect is due to an occlusion caused by a leaf overlapping a bud in region C3 and C4. Since the exact location of the bud cannot be determined from the image data, its location can be estimated. Alternatively, if an active vision system is being used, a new data set can be acquired from a different perspective thereby providing additional information about the region. Resolving this defect completes the task-focused modeling process.

To complete the cutting task, the high confidence buds are connected along the plant axis into a single graph. Figure 6 shows the resulting model with the final cutting sites identified and marked by vertical lines.

Figure 2 - Region of interest determined in iteration 1.

Figure 3 - Model and new regions of interest determined in iteration 2.

Figure 4 - Model and new regions of interest determined in iteration 3.

Figure 5 - Completed model determined in iteration 4.

Figure 6 - Final cutting sites.

We have seen how task-focused modeling can be used to efficiently decide the cutting sites along a plant. In summary, modeling defects are found and a corresponding region of interest is identified in the image data. The region of interest specifies part of the image data where additional information can be extracted to resolve the modeling defect. Finally, when the modeling process is finished, the task of the machine vision system is executed.

In the next section, we generalize the plant cutting procedure described above to produce a scheme for task-focused modeling.

A SCHEME FOR TASK-FOCUSED MODELING

We begin by defining task-focused modeling. A machine vision system's *task* is its job, purpose, or goal. The *effort* expended in performing the task is measured in terms of processing time, steady state error, input energy, processing resources, etcetera. A *model* consists of both information and a vessel that stores the information. *Task-focused modeling* is an adaptive process that expends a minimal amount of effort in constructing a model having sufficient detail to facilitate the completion of the task.

Task-focused modeling has the following properties:

- 1.) The model contains information needed to cope with the problems of unreliable or insufficient data.
- 2.) The effort expended in constructing the model is minimal, thereby conserving time and system resources.
- 3.) The model captures those aspects of the objects in the scene that are useful in carrying out the specific task. The type of information stored in the model simplifies further reasoning about the particular task.
- 4.) Modeling defects are resolved by deciding the type of defect, a region of interest in which further information can be extracted, and an appropriate information extraction algorithm. This focuses the processing effort on those regions of the data that contain information useful for the completion of the task.
- 5.) The model can be abstract or physically correlated with the objects in the scene depending on the requirements of the task. The nature of the task and the image data places constraints on the type of model and information extractors used in the task-focused modeling system.

Figure 3 illustrates a scheme for task-focused modeling. Our scheme consists of three modules:

Information-Extractor/Model-Constructor, Model Analyzer, and Task Execution. The components in this scheme and their purpose are discussed below.

Figure 7 - A Scheme for Task-focused Modeling.

2.1 Information-Extractor/Model-Constructor module.

The Information-Extractor/Model-Constructor consists of independently operating global and local feature extractors. The feature extractors are applied to specific regions of interest in the image data thereby producing additional information for resolving the associated modeling defect. The Model Constructor adds this new information to the model.

2.2 Model Analyzer module.

The Model Analyzer detects and identifies modeling defects in the model. The detection of these defects is based on an a priori database of modeling rules. When the Model Analyzer discovers a defect in the model, it performs several functions:

- 1.) If the real-time acquisition of new data sets is an option, then the Model Analyzer controls the data acquisition process.
- 2.) If several data sets are available simultaneously, the Model Analyzer selects an appropriate data set from which additional information can be extracted.
- 3.) The Model Analyzer finds regions of interest in the image data where additional information can be extracted to resolve the corresponding modeling defect.
- 4.) The Model Analyzer selects the next information extraction algorithm to apply to a region of interest based on the type of defect in the model.

A model is *fit* when it contains sufficient information for the task of the machine vision system to be

completed. When the model is fit, the iterative modeling process ceases and a "Model Complete" signal is issued to the Task Execution module.

2.3 Task Execution module.

When a "Model Complete" signal is issued, the Task Execution module uses the completed model to carry out the task.

In summary, the Information-Extractor/Model-Constructor and Model Analyzer perform iterative modeling of the objects in the scene. When the modeling process is complete, the Task Execution module uses the model to complete the specific task of the machine vision system.

CONCLUSION

In this paper, we described a scheme for task-focused modeling in machine vision. The task-focused modeling procedure produces a model that is detailed in regions of interest to the task and is less detailed elsewhere. Task-focused modeling is especially useful when the objects being processed are nonrigid and occur in irregular shapes and sizes. In these cases, the location and type of modeling defects vary from image to image thereby changing the location of the regions of interest in the image data. In addition, the sequence of information extraction algorithms required to resolve modeling defects also varies from image to image. We illustrated our procedure for task-focused modeling in an application to clonal micropropagation in automated agriculture.

5. ACKNOWLEDGMENTS

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6. REFERENCES

- 1.) Y. Aloimonos, "Purposive and qualitative active vision," *Proceedings of the 10th International Conference on Pattern Recognition*, pp. 346-360, June 1990.
- 2.) R. Bajcsy, "Active perception," *Proceedings of the IEEE*, 76(8), August 1988.
- 3.) B.K.P. Horn, Robot Vision, The MIT Press, 1989.
- 4.) K. Stonecipher, Industrial Robotics, Machine Vision, and Artificial Intelligence, Howard W. Sams and Company, 1989.

Chapter 9 SUMMARY

In light of the objectives, the achievements of this project can be divided in four groups:

a. Biology

1. The main biological objective of this research project was to generate plantlets with uniform morphology, suitable for automated mass micropropagation, via classification by machine vision. Ancymidol was used to regulate plant growth and enhance chlorophyll synthesis. It was found that ancymidol enhances the color contrast between leaves and stems. Plantlets treated with ancymidol generated more axillary buds per plantlet than the control.
2. Machine vision was used to evaluate the morphological changes which resulted from the biological experiments. Geometrical features such as number of buds or internode length can be precisely measured and automatically logged. Some important features that till now could only be observed qualitatively, can also be measured, e.g. absolute color and color contrast. Additionally, physiological features like weight of microtubers can be accurately estimated from automatically measured geometrical features of the plantlet's image.

b. Engineering – MACHINE VISION

1. We developed a specialized algorithm for extracting geometric features of potato plantlets, which takes advantage of the inherent difference between the geometrical shape and gray scale levels of the stems and the leaves as well as of the rather simple connectivity rules of attachment between them.
2. Using color machine vision and a Neural Network based classifier, we have laid a basis for a practical system, which may be used for automatic classification of tissue culture segments of potato plantlets. Instead of the conventional use of black and white cameras and geometric features only, we used color features in combination with geometric features, while taking advantage of the hardware implemented color frame manipulation capabilities, which are now available in most commercial imaging boards. This facilitates high speed plantlet classification by automatic subculturing systems.
3. By using geometric features only, we were able to achieve a total mean misclassification rate of 12%. Similarly, by using color features only we obtained a misclassification rate of 7%. The misclassification rate was further reduced to 5%, by combining geometric and color features, while using a Neural Network classifier.
As this misclassification rate is still too high for practical implementation, we developed a classifier voting algorithm that combines the decisions of several classifiers in order to reduce the classification errors further. Classification tests with this algorithm showed that the potato shoots can be classified with a misclassification rate of about 1.4%. This result is exceptionally good, given the extremely high variability of the inspected product.

4. A scheme for task-focused modeling was developed. The task-focused modeling procedure produces a model that is detailed in regions of interest to the task and is less detailed elsewhere. The effectiveness of this procedure was demonstrated on potato plantlets.
5. We developed a technique for selecting the best colored illumination condition for maximizing the contrast between objects when the spectral reflectance $s(\lambda)$ of the objects in the scene and the camera's spectral response curves are known. Experiments on potato plantlets confirmed the effectiveness of this technique for finding colored illumination that maximizes the contrast between the leaves and stems.

c. Graduate students

Two Master's level and one Doctoral level graduate research thesis were completed. One Master thesis by Oded Cohen at the Technion, and the other by Mark Vriesenga at UCI. The Doctoral thesis was completed by Victor Alchanatis at the Technion.

The first Master thesis was an initial study on identifying propagation organs, in static images of potato tissue culture plantlet segments, entitled:

"Machine Identification of Buds in Images of Plant Shoots" .

The second Master thesis dealt with the development of optimal illumination for efficient machine vision and classification of tissue cultures, entitled:

"Colored Illumination for Maximization Contrast in Machine Vision".

The doctoral thesis dealt with further development of efficient high speed algorithms for machine vision and precise classification of manually pre cut plantlet segments, entitled:

"Machine classification of potato shoots for automatic micropropagation"

d. Papers and presentations at International Symposia

1. Alchanatis V, Peleg K, Ziv M and Cohen O, 1991. Geometric and color features for tissue culture classification. Proceedings of 8th Israeli Symposium on Artificial Intelligence and Computer Vision, Kfar Hamacabia Ramat-Gan, pp. 205-221.
2. Vriesenga M, Healy G, Peleg K, Hoffman F and Sklansky J, 1991. Colored illumination for enhancing Discriminability of objects. Proceedings of 8th Israeli Symposium on Artificial Intelligence and Computer Vision, Kfar Hamacabia Ramat-Gan.
3. Alchanatis V, Ziv M and Peleg K, 1992. Morphological control and mensuration of potato plantlets from tissue cultures. The Annual Israeli Symposium on Plant Tissue Cultures and Plant Molecular Biology, Beit Dagan, Israel.
4. Vriesenga M, Peleg K and Sklansky J, 1992. Task focused modeling in automated agriculture. SPIE conference, San Diego 1992.

5. Alchanatis V, Peleg K, and Ziv M. Classification of Tissue Culture Segments by Color Machine Vision. (Accepted for publication in the Journal of Agricultural Engineering Research).
6. Alchanatis V, Peleg K and Ziv M. Morphological Control and Mensuration of Potato Plantlets from Tissue Cultures. (Submitted for publication).
7. Vriesenga M, Healy G, Peleg K and Sklansky J. Colored illumination for enhancing discriminability in machine vision. (In preparation).
8. Alchanatis V and Peleg K. Pattern Recognition by Voting Classifiers. (In preparation)
9. Vriesenga M, Hoffman F, Peleg K and Sklansky J. Tailoring plants for improving segmentation in machine vision. (in preparation)
10. Peleg K., Cohen, O., Ziv M. and Kimmel E. Machine identification of buds in images of plant shoots. Accepted for publication in Machine Vision and Applications. (in press)

Recommendations for practical implementation

The results reported here indicate that plantlets cultured in 0.25ppm ancymidol provided the best plantlet morphology for automated mass micropropagation and the highest propagation potential. Additionally, it seems that ancymidol has a cumulative effect on the growth regulation system, that is expressed by a shorter period for microtuber formation.

Using machine vision which combined color and geometrical characteristics of the plantlet segments and a classifier voting algorithm we have laid a basis for a practical system, which may be used for automatic classification of tissue culture segments of potato plantlets. Algorithms for color and geometric feature extraction provided the features that were used by a classifier voting algorithm that was developed and applied on potato shoot segments. We found that the combination of a Neural Network based classifier with a 3 Nearest Neighbor classifier and a Maximum Log-Likelihood classifier resulted a contamination index of as low as 1.37%, which is essential for the commercial application of such an automatic system.

Recommendations for further research

For commercial implementation of an automatic system for micropropagation, more work has to be done in several directions:

1. The cumulative effect of ancymidol on plantlet morphology has to be studied. So far, the results show that the morphology of the plantlets of the second generation grown in ancymidol, remains the same.
2. Sterility must be maintained throughout all the stages of the process, including the automatic

chopping stage. Instead of designing an apparatus similar to those used by human workers (sterilized scalpels by immersion in alcohol and burning), a laser beam may be used as an alternative cutting means. This was demonstrated by our initial small scale experiments. Further work has to be done on the various biological and engineering aspects of automatic chopping by a laser beam.

3. In the suggested automatic system, the shoot segments are dropped into the containers, instead of being manually placed into the nutrient medium. First generation plantlets thus grown, show no ill effects. The effect of random dropping on consecutive grown generations of the plantlets has yet to be determined.

4. Implementation of a prototype conveyor system.

The developed machine vision and classification algorithms were so far tested on stationary images. The implementation of a conveyor prototype will enable the evaluation of these algorithms with images acquired while the plantlet segments are moving by the camera.

DESCRIPTION OF COOPERATIVE RESEARCH ACTIVITIES AND RESULTS.

Extensive experiments on potato plantlets, have been conducted both in Israel and in the US, in an effort to develop the optimal plantlet morphology, by varying nutrient compositions, and environmental conditions, e.g. various nutrient recipes, lighting intensities and duration, temperature and partial growth in complete darkness.

Variations in chemical composition of the nutrient media particularly large scale experiments with Ancymidol on several generations of plantlets were conducted by the Israeli team.

Environmental variations such as different lighting conditions and temperatures in growth containers, were conducted in the US, while using the optimal nutrient media compositions developed in Israel.

The main machine vision task of the Israeli team was development of efficient high speed algorithms for color machine vision and precise classification of manually pre cut plantlet segments, i.e. identifying propagation organs, in images of pre-cut potato tissue culture plantlet segments with very high accuracy.

The main machine vision tasks of the US team were:

1. Optimizing the illumination system, which is essential for efficient color segmentation, i.e. for maximal color differences between the background of the image, the leafs and stems of the plantlets.
2. Generating efficient high speed algorithms for finding optimal cutting sites on whole potato plantlets, as a preamble to subsequent automatic plantlet chopping.

Continuous exchange of information by E-mail and FAX messages between the two graduate students, Mark Vriesenga at UCI in the US and Victor Alchanatis at the Technion in Israel, were very helpful in minimizing duplicative efforts. Similar communication between the principal investigator Kalman Peleg and all cooperating investigators in Israel and in the US, facilitated coordination of efforts throughout the project. Discussions during the mutual visits of the Israeli investigators in the US and both cooperating investigators from the US in Israel further facilitated cooperation and coordinated distribution of efforts. Of the ten papers and presentations at international symposia, which resulted from this project, four were prepared jointly by the principal investigator in Israel and the investigators in the US.