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Texas Journal of Microscopy

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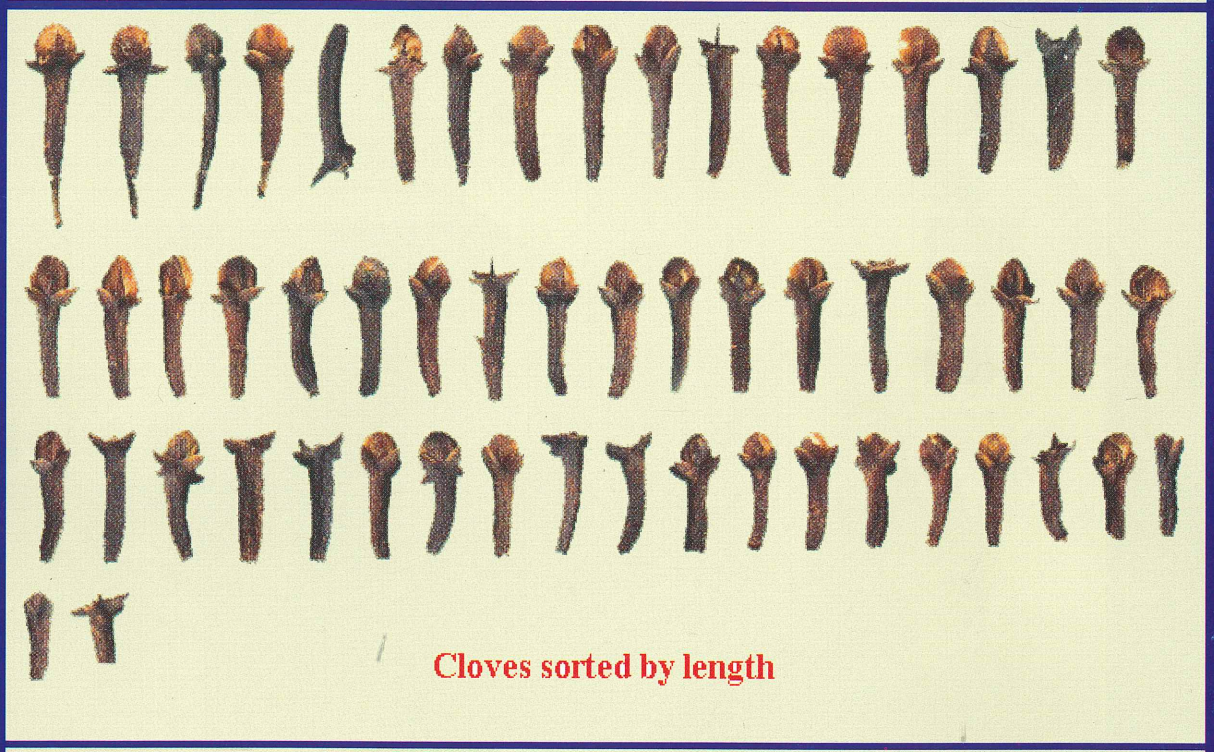
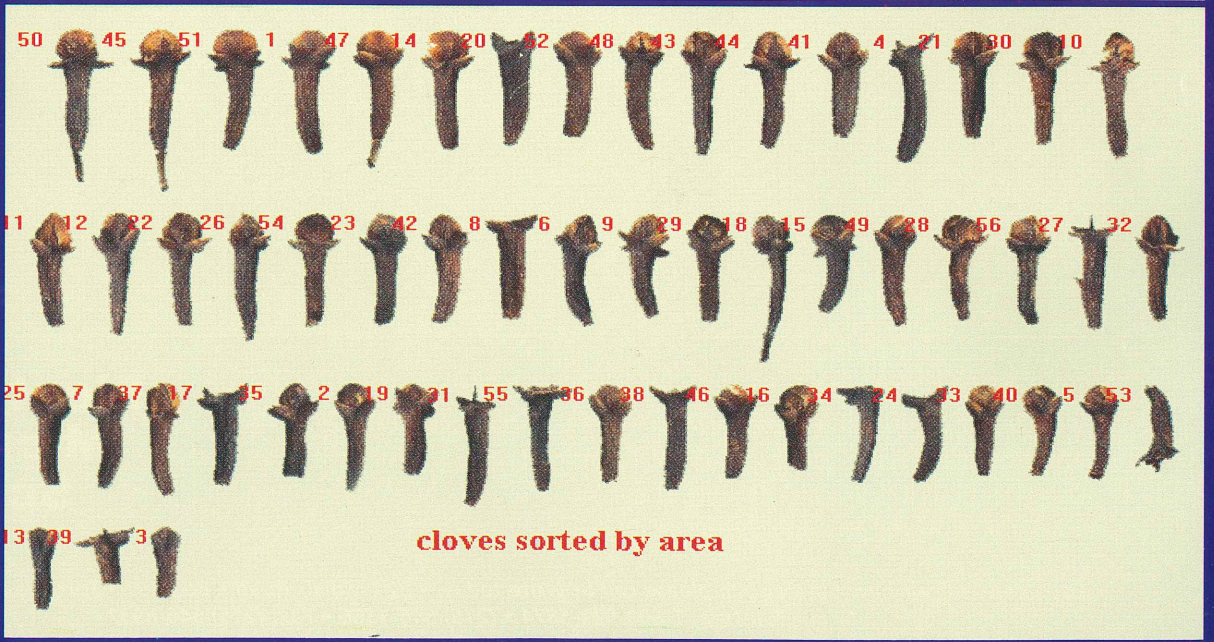


Cloves sorted by length



cloves sorted by area

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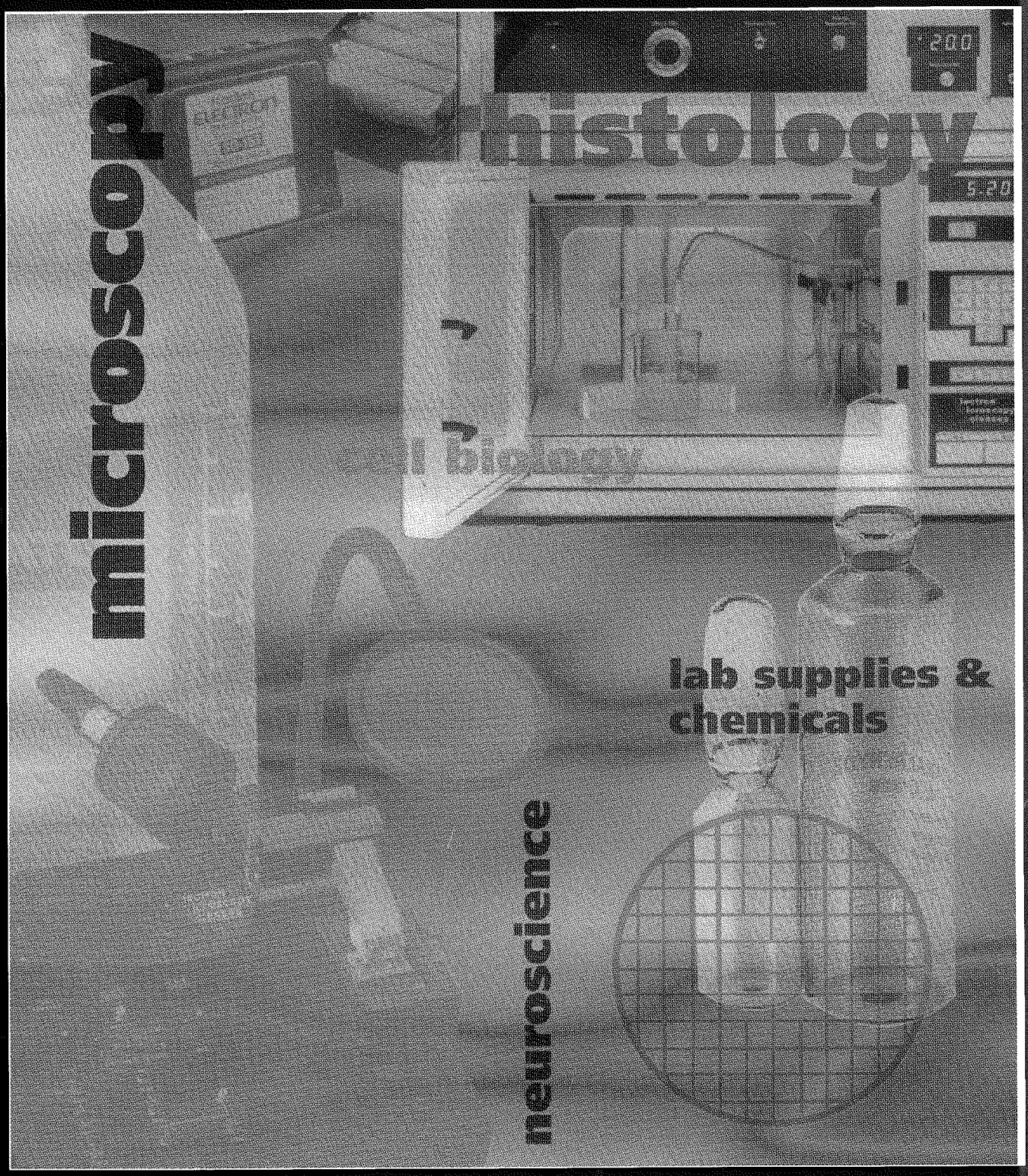

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VOLUME 31, NUMBER 1, 2000
ISSN 0196-5662

David C. Garrett, Editor
Department of Biological Sciences, University of North Texas, Denton, TX 76203

Official Journal of the Texas Society for Microscopy
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ON THE COVER
Three images of cloves, the dried flowers of *Eugenia aromatica*. Upper panel shows unsorted scan of cloves. The middle panel shows cloves sorted by length. The lower panel shows cloves sorted by area and numbered according to their original position in the count program. See "Use of sorting programs in the examination of food products" contained in this volume for more information.
Howard J. Arnott, Dept of Biology and Center for Electron Microscopy, Univ. of Texas at Arlington, Arlington, TX 76019

President's Message

First, let me express my appreciation to all who participated in our Fall 1999 Meeting, October 28-30 in Houston. Dennis Ward from the FBI Microanalysis Laboratory in Washington, D.C. delivered an invited paper entitled "SEM and EDX Analysis in Forensic Science." We were also exposed to the use of light microscopy in a forensic lab by Steve Robertson from the Crime Laboratory Service of the Texas Department of Public Safety in Austin. Our third invited speaker was Dr. Bruce Mackay from the University of Texas M.D. Anderson Cancer Center in Houston. Dr. Mackay, a long time participant and supporter of TSM, presented a series of exquisite micrographs during his presentation, "Diagnostic Electron Microscopy, Past and Present." Our workshop was put on by Mike Davis, Vijian Dhevan and Dan Saban from Nikon, Inc. They gave a very informative presentation and provided hands-on instruction in techniques for the optimization of the light microscope. Finally, I would like to thank the TSM members who presented their research in a total of 14 platform presentations, and our corporate members who exhibited at the conference and helped to sponsor our Thursday social and meeting breaks.

This journal will be published in conjunction with TSM's participation in SCANNING 2000, May 9-12 in San Antonio. Fourteen presentations by TSM members are on the program. Our members elected to merge their talks into the general sessions of this twelfth annual international conference sponsored by the Foundation for Advances in Medicine and Science (FAMS) and SCANNING, The Journal of Scanning Microscopies. Our sincere appreciation goes out to SCANNING/FAMS for providing registration waivers for student volunteers, student scholarship awards, and exhibit and meeting space for TSM. Mary Sullivan, Tony Bourgholtzer and Paula Pivnick have been especially helpful in arranging for TSM's participation in the conference and deserve special thanks. I hope this experience will be a positive one for those who have the opportunity to attend, and that exposure at this international venue will result in increased participation in future TSM meetings.

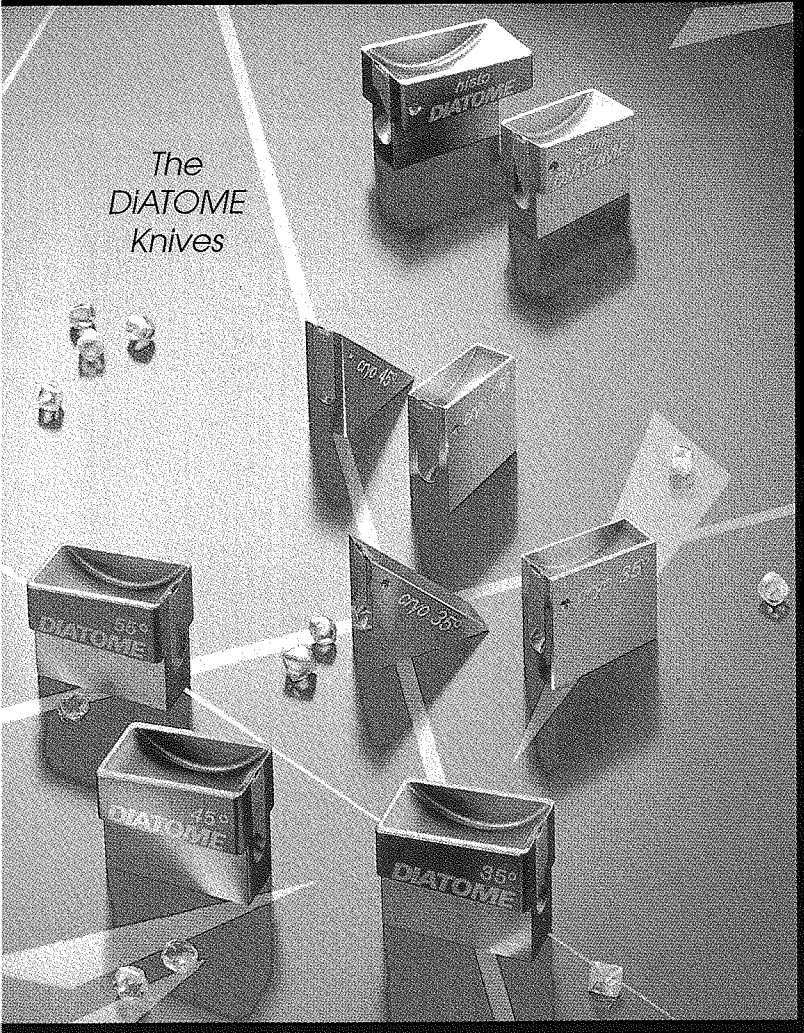
The Fall 2000 Meeting of TSM will be held October 26-28 in Dallas. Incoming Program Chairman Pam Neill and Kevin Cronyn of NSA Hitachi have been working on a workshop series for October 26 to be held at Texas Instruments. Topics to be covered include basics of SEM, low voltage, and environmental SEM. We appreciate their efforts and encourage you to make plans to attend and present your research at the fall conference. Please contact Pam or any member of the Executive Council with your comments and suggestions.

In closing, I would like to thank all those who have provided support and guidance to me over the course of my year as TSM President. I would like to extend special thanks to outgoing officers Bob Droleskey, Jonnell Beaird and John Williard for all of their help. As we welcome a new Executive Council team and they start their year of service, please assist them by communicating your suggestions on future meetings, workshop topics, and invited speakers. Your input is needed for TSM to thrive and grow.

Sincerely,

Josephine Taylor
TSM President, 1999-2000

Treasurer's Report unavailable at press time



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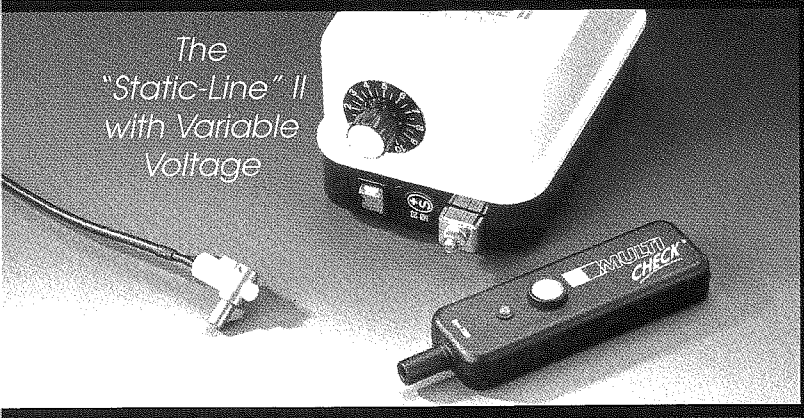

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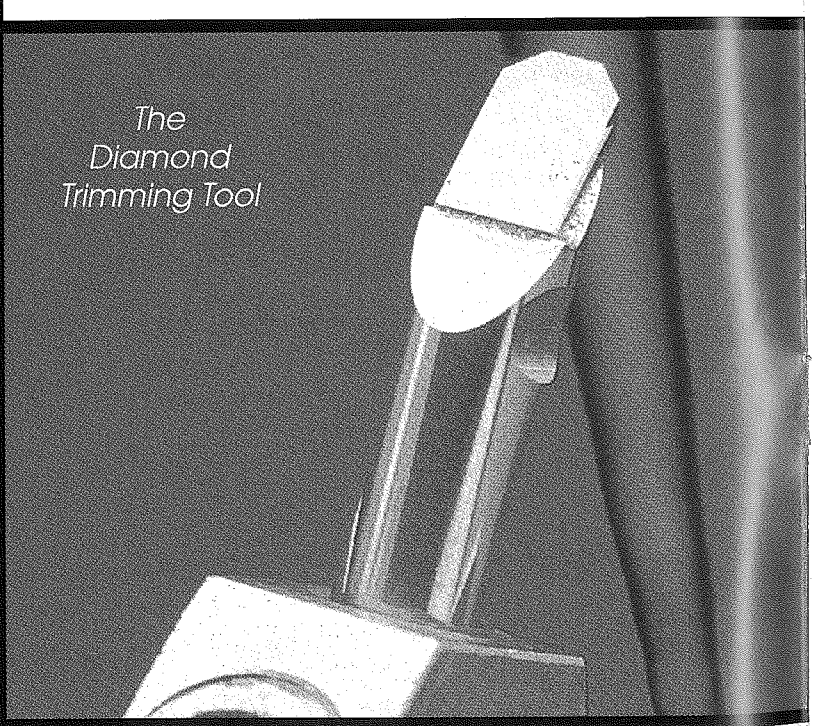
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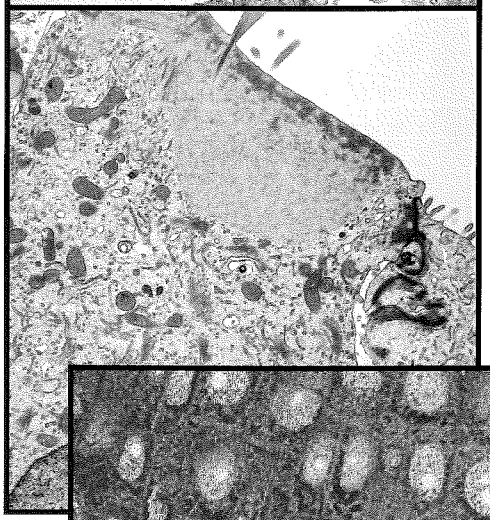

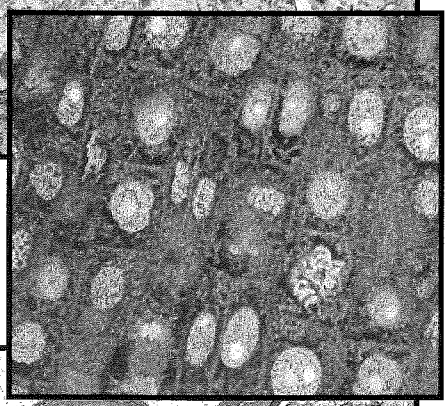

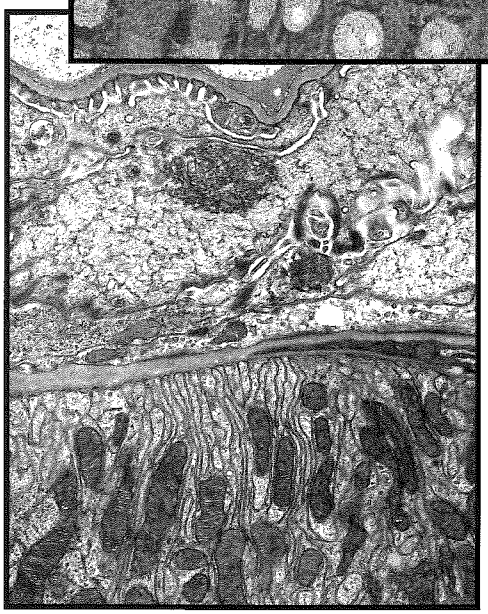

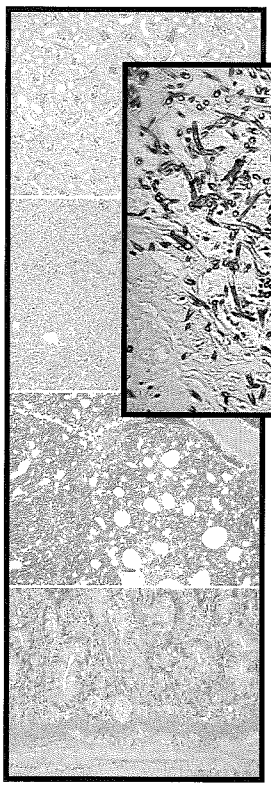

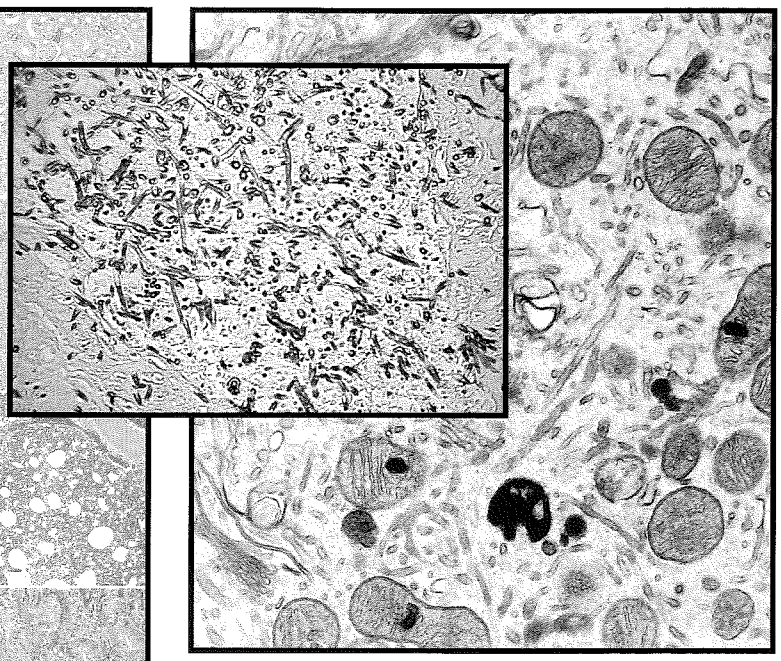

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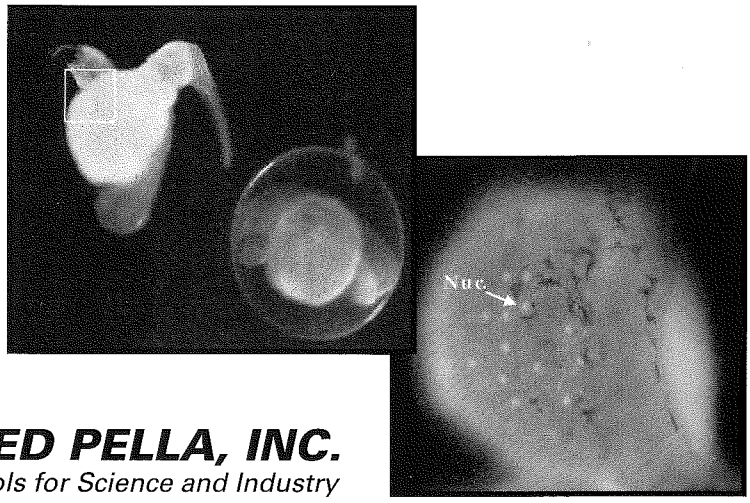

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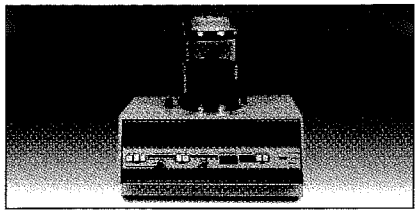

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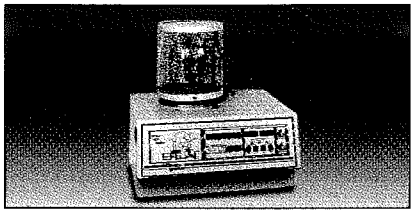

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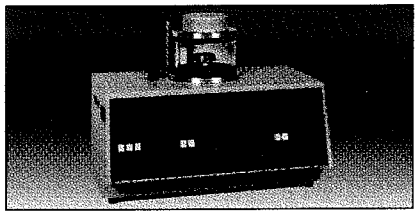

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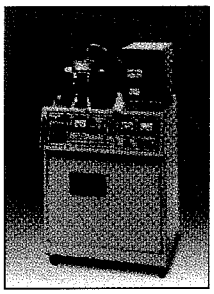

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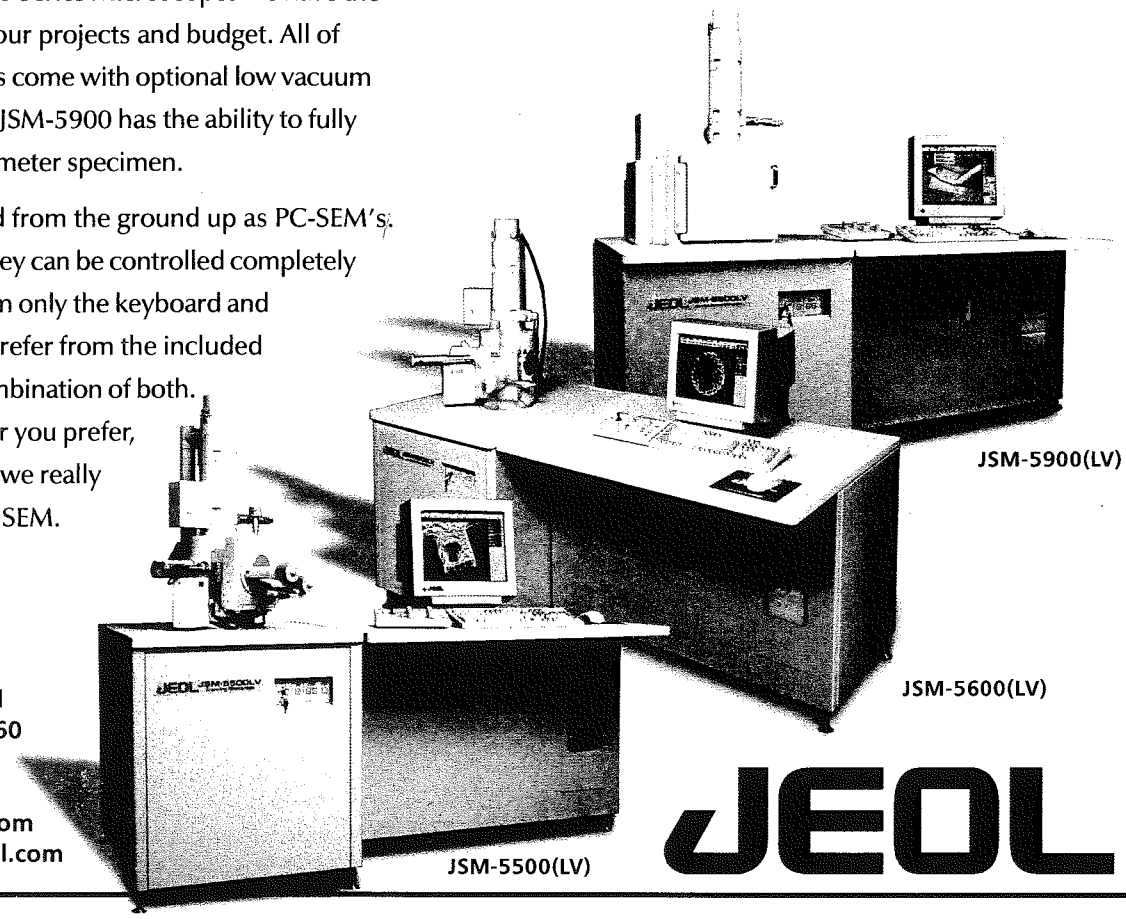

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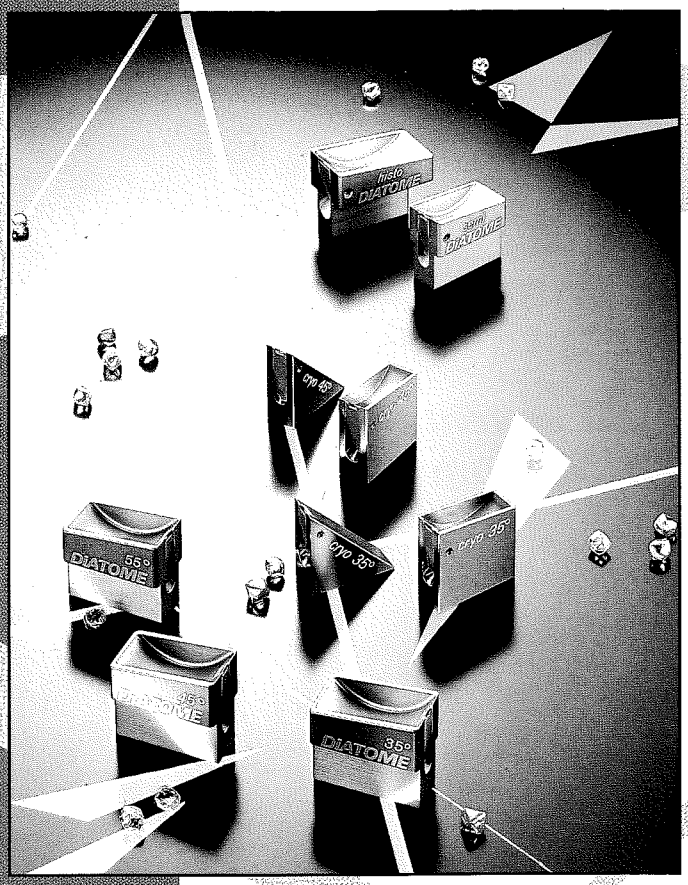

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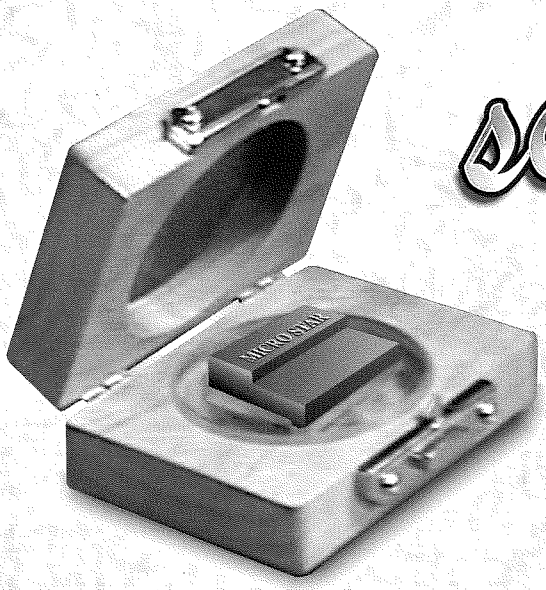

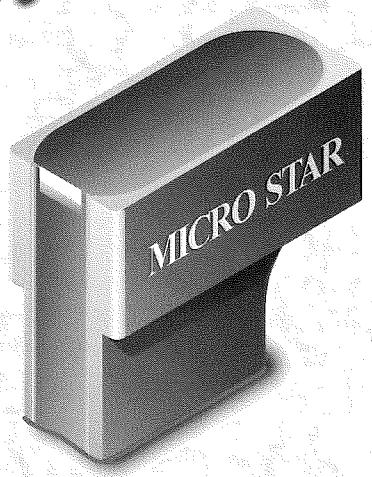

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REFERENCES to other work should be consecutively numbered in the text using parentheses and listed at the end, as in the following examples:

(1) A. Glauert, Practical Methods in Electron Microscopy. Vol. 2 (North-Holland. Amsterdam, 1974) 82-88.
(2) P.S. Baur, Jr., G.F. Barratt, G.M. Brown and D.H. Parks. Ultrastructural Evidence for the Presence of "Fibroclasts" and myofibroclasts" in Wound Healing Tissues. J. of Trauma. 19 (1979) 774-756.
(3) D. Gabor. Information Theory in Electron Microscopy, in: Quantitative Electron Microscopy. Eds. G.F. Bahr and E. Zeitler (Williams and Wilkins, Baltimore, 1956) 63-68.

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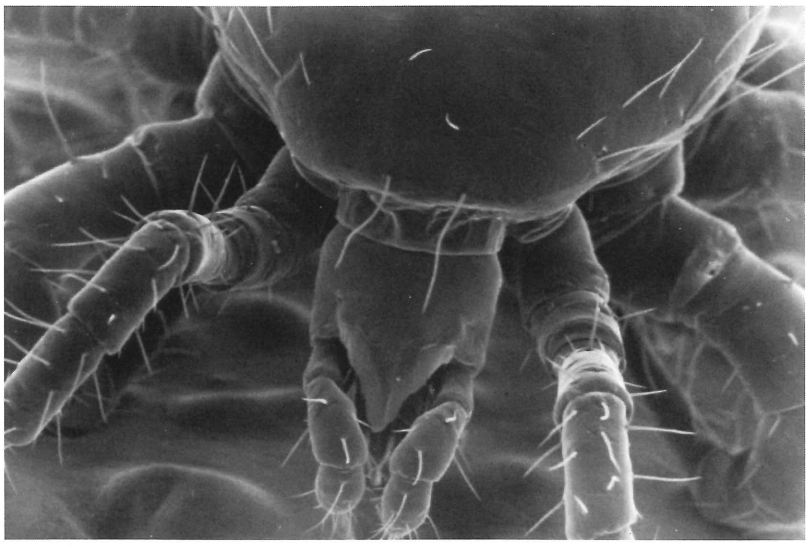

The micrograph on the back cover of Volume 30, Number 2, p. 56, 1999, is a mite that was removed from the cervical membrane of an adult beetle, *Hypothyce mixta* Howden (Scarabaeidae). This dorsal view shows the gnathosoma which consists of the pedipalps, chelicerae, and the chela. Only the pedipalps can be seen dorsally. The two pair of front legs and their setae can also be seen.

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USE OF SORTING PROGRAMS IN THE EXAMINATION OF FOOD PRODUCTS

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ABSTRACT

Modern computers and robust image analysis programs provide useful options for microscopists and food scientists. This paper will introduce readers to the potential utility of image analysis sorting algorithms in food and natural sciences. Programs that are able to provide visible information by sorting the objects under consideration offer easy solutions for the comparison of commercial food products. The paper illustrates several sets of sorted objects and indicates some of the methods and problems of gathering and presenting data from sorting programs. A comparison between slots and sorting is made.

INTRODUCTION

A number of image analysis programs which can aid in the examination of small objects have become available to microscopists (1-6). Image Pro Plus, Optimus, Sigma Scan Pro and MetaMorph represent a few of the programs currently available for utilization. Coordinated with the development of these programs the raw power of desktop computers has expanded rapidly. At the same time the ability to collect digital images from either light or electron microscopes has also advanced swiftly. The improvement of computers, digital cameras and image analysis software has combined to provided new research opportunities for scientists. Food scientists have joined in the use of these tools and the acceptance of such studies is becoming more and more visible. Papers using image analysis as a part of the examination of various food products are now common (1-6). Recently the image analysis software had been augmented by the addition of *sorting programs* which allow the concise viewing of a variety of structural components. In programs, such as Image Pro Plus (4.0), the sorting of components in a visual manner can be conveniently carried out. When sorting programs are applied to the study of small food structures such a grains, spices, pepper, and salt, they can provide both visual and quantitative results. Of course these sorting programs are not limited to small objects. They can also be applied to larger food and non-food structures. "Apples and oranges, nuts and bolts, ships and sealing wax, flotsam and jetsam" can easily be analyzed and the data presented in a concise visual representation. While the sorting programs are broadly useful, I will be concerned with only how image analysis sorting programs, not image analysis in general, can be applied to some common small food products.

MATERIALS AND METHODS

The analyses reported here were made using Image Pro Plus (4.0) software running on a Gateway PC. The images used were produced by directly scanning seeds and other small objects on a Hewlett Packard Scan Jet 4200C into Photoshop (5.5). Microscopic objects, such as pollen, were examined with a Spot digital camera, large objects such as leaves can be photographed with a digital camera, such as the Kodak DC 290. Whatever the origin of the images, they were analyzed by Image Pro Plus (4.0) software. When scanning these small objects, I laid them on a transparency film placed on scanner surface to protect it. This was convenient, not only because the scanner bed is protected from the materials but also when the scanning process is through the objects can be lifted off the scanning bed without difficulty. By slightly bending the transparency sheet it was easy to replace small seeds, spices and other objects in their containers. The method of scanning varies depending on the nature of the objects being scanned. In order to get proper scans of some objects it may be necessary to use alternative backgrounds to that of the ordinary white background which is found on most scanners. I found that black, blue and even green backgrounds sometimes produced quality images. Another problem that occurs when scanning small objects with background lying directly on the objects is the production of objectionable shadows. The shadows make it difficult to properly threshold the individual objects. When shadows occur, I found that rasing the background some distance above the objects (3 to 20 mm) or removing the background entirely (the room then becomes the background) was useful; trial and error seems to be the best way to determine the proper procedures for each case. Similar difficulties in thresholding can occur when the objects have bright spots (for example shiny seeds) or when the object has compo-

nent color(s) that is similar to the background (Fig. 3). Care in scanning can reduce most of this problem. It is also important to place the individual objects so they don't touch each other. In many programs touching individuals can be separated using program features that "cut" or "separate" two particles, however, this makes additional work for the operator. Calibration was easy to establish by using the image of a 10mm grid scanned at the 300 dpi the same dpi at which my samples were scanned.

RESULTS AND DISCUSSION

Some examples of sorting using the Image Pro Plus (4.0) sorting program are presented in Figs. 1-6 and on the cover. The upper panel on the cover shows a typical scanned image in which cloves (dried flowers of *Eugenia aromatica*), purchased at a local food market, have been placed on the scanner in a random manner but with care so that they do not touch each other. The major problem in obtaining scans like the upper panel arises from the relatively dark characteristics of the cloves resulting in a scan in which the brightness and contrast had to be modified in Photoshop. Once an acceptable image was obtained the objects were counted using the count and size menu. Following satisfactory count and measurements of the parameters of the particles specified, the object sort program could be used. In the case of the cloves the object sort program worked very well without thresholding. Note, in some cases thresholding is an absolute necessity for obtaining satisfactory measurements and counts necessary prior to sorting. The cover's middle panel displays an image of the same assemblage of cloves, however, in this image they were alined and ordered according to their length through use of the object sort program. I also obtained images in which cloves were sorted by width, area, and optical characteristics. It is also possible to obtain sorted images in which one or more of forty other factors could be used for sorting. The bottom panel of the cover illustrates the same group of cloves sorted by area. In this case I left the numbers, which are assigned to the individual cloves visible; they are printed in red. This is another useful feature of the program that lets you follow individual cloves (objects) throughout a series of different measurements and sorts. These coordinate with the data spread sheets so that a series of characteristics of each of these objects can be obtained. Examination of the cloves shows that the two ends of the flower are very different. The sort program in Image Pro Plus (4.0) has an option to aline the long axis of an object either vertically or horizontally. Obviously these were alined vertically, but the program also provides an option to reverse the up or down orientation of the objects thus making it possible to have all the cloves oriented the same way, that is, with the peduncle down and the bud facing upward. This is a mechanical operation requiring the direction to be set by the operator. Note that in row one of the middle panel the operator misorientated the fifth clove.

An image of lima beans (*Phaseolus vulgaris*) sorted by area is presented in Fig. 1. Obtaining a satisfactory scan for the lima bean count/sort procedures also presented some difficulties. It was easy in this case to have the edges of the lima beans partly eroded (see the left bean in the lower row as an example). Scanning lima beans was also complicated because of their color. Grey/white does not stand out against a white background and on the other end of the scale it is also difficult because their white color contrasts very strongly with a dark or black background. Note that the last "bean" in the lower row is only a fragment of an entire bean. The program will not eliminate particles that are within the parameters set in the measurement definition. However, the program will allow the operator to eliminate any "beans" (objects) that do not seem appropriate to the study group.

Figure 2 illustrates how exquisitely a simple food item such as *rotini*, a macaroni product, can be evaluated though the use of object sorting. The length of the individual *rotini* clearly varies, the diameter is more uniform. Using other components of an image analysis program we could determine the mean and variation in length (or width) for this sample. Having compared the members of one sample, a study could be easily expanded allowing us to compare several samples (different containers, different batches, different producers, etc.). Through such an investigation one could quickly learn about the variation both within samples and between samples. I used object sorting with out difficulty to examine the idiosyncratic variation in commercial oat products (6) (Figs. 5 and 6).

A mixture of navy and pinto beans (*Phaseolus vulgaris*) sorted by using optical density as a sorting factor are shown in Fig. 3. The sorting procedure cleanly separated the two bean types except for two errant navy beans in the fifth row. Pinto beans illustrate an exaggerated example of the same problem that I encountered with the lima beans, namely erosion of the edges. However, in the pinto bean the erosion was much more substantial and bothersome. With optical density the sorting occurred without problem, however, sorting using area as a factor would not have been correct for the pinto beans. This problem seems to be due to the fact that pinto beans have two colors and the colors are mixed differently in each bean. One presumes that the same problem would exist if one were using images of tigers or zebras. Another interesting thing to note in Fig. 6 is the fact that in the seventh row there are a large number of very small particles, fragments of the bean coat, dust, etc. These can be eliminated by placing a specific lower size limit for the particles which you wish to count. When done, particles

smaller that the lower limit are eliminated. I have addressed the problems of scanning objects like cloves, lima and pinto beans but I want to emphasize that many small objects can be scanned without any trouble. When trouble occurs changing scanning parameters usually will help. Modifications of the images with Photoshop prior to loading them an analysis program also can be of considerable help.

Another feature of the sort object program in Image Pro Plus (4.0) is illustrated in the image of Fig.4. Four groups of cloves (objects) have been color designated according to their area dimensions. This is done by designating four bins, each which are assigned a color code. Here dark blue is assigned to those cloves with the largest area and red to those with the smallest area, etc. The number of bins is set arbitrarily and can vary from one to ten. Creation of a series of these "pictograms" can effectively characterize the apparent randomness found in many samples like cloves, etc.

Images of quick oats are shown before and after sorting in Figs. 5 and 6. Examination of Fig.5 shows that quick oats are made up of broken pieces of rolled oats whose size varies quite dramatically. It is of course this variance in size, especially the large number of smaller sized particles, that is responsible for the "rapid" cooking of quick oats. Fig. 6 shows the wide variation of the sorted pieces of quick oats. The sorting of large and small particles is quite good, however, there are several cases where individual particles were touching and hence they were treated as single objects. There are seven cases of this in the top row and a few cases in each of the seven successive rows. Careful operator manipulation of the original image would have eliminated this problem. For example, the third "double grain" seen in line two from the top can be found in the lower left third of Fig. 5. Clearly, this "double" merely represents the juxtaposition of two grains in the original scan. There are several ways this could be avoided. One, you could carefully separate all the grains so that none are touching, however, in this case it is not so easy to accomplish. The second method involves the operator separating (cutting) doubles into individual particles as they are viewed on the computer screen. Many image analysis programs have components which automate the process of cutting objects apart. A third method would be to set the size of the largest particle, as separately measured, as the upper limit for particles to be accepted into the sort. Never the less, in the end the operator is responsible for examining their images for such flaws. Data derived from images with "double" or " triple particles" in may be useless and negate any such investigation.

In conclusion, it seams clear that sorting programs may be of substantial use in the examination of food and other commercial products as well as many other "natural objects." I believe that groups of spores or pollen grains from many different species could easily be counted and separated using these sorting programs. This kind of automation allows the collection of data with relatively little expenditure of time and with substantial confidence in both the resulting data as well as the images that are produced. My reaction when I first saw a sort program in action was almost exactly like that of winning a $1200.00 slot machine jackpot in Deadwood, SD.

ACKNOWLEDGMENTS

I thank Mike Davis for first pointing out sorting programs and thank my recent image analysis class members, Mike Davis, Debra Dixon, Steve Mercer, Joel Montgomery, Cindi Schwartz, Wanda Shotsberger, Sandra Westmoreland, Russell Wier, Clay Williams, Ashley Worlock, April Zoccali, and Tim Henry for their discussions of the intricacies of Image Analysis. I thank Tim Henry, Sandra Westmorland, Cindi Schwartz, and Jean Arnott for help in preparing the manuscript.

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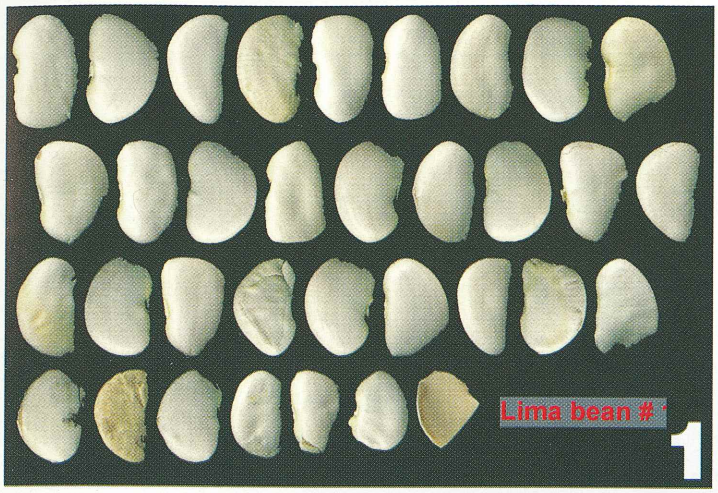

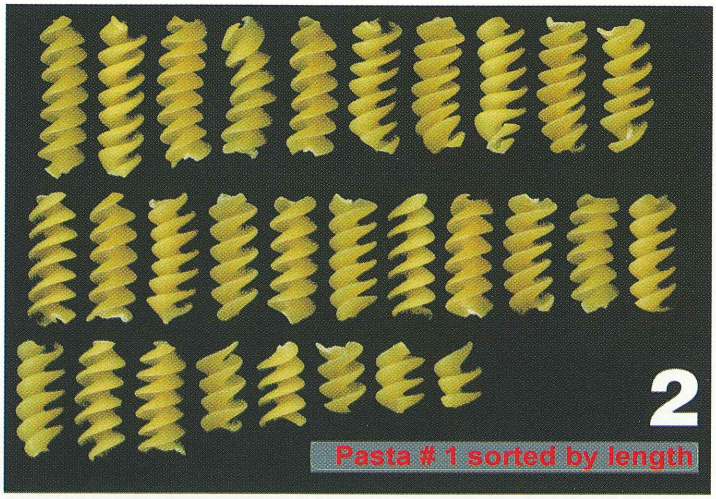

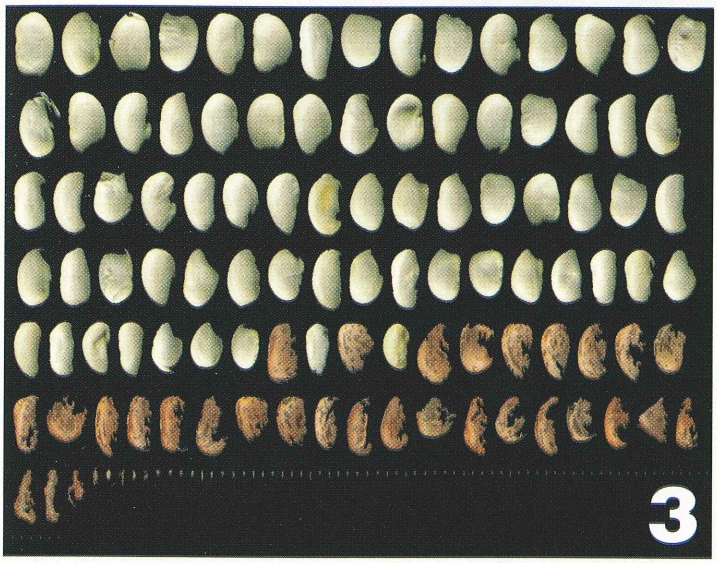

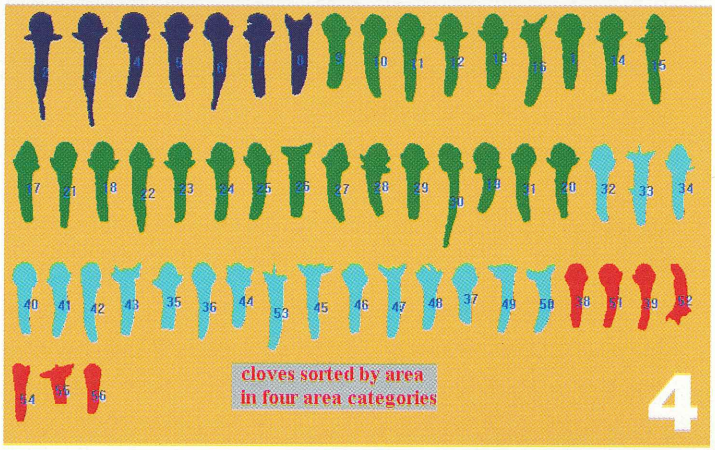

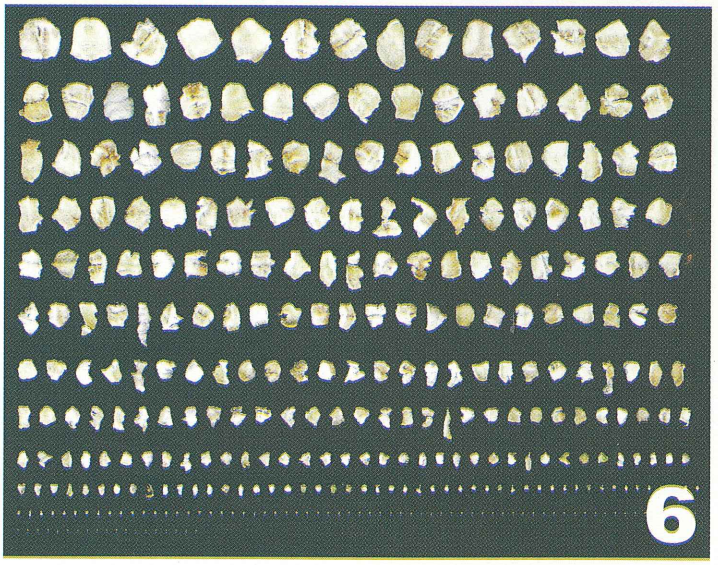

FIGURE LEGEND.

Figures 1-6. Images produced using Image Pro Plus (4.0). **Fig. 1.** Lima beans sorted by area, see text for scanning problems. **Fig. 2.** Rotini sorted by length. These pasta pieces were easily scanned, counted and sorted. **Fig. 3.** Navy and pinto beans sorted by IOD (integrated optical density) characteristics. Note vignetting in both types, but especially in the pinto beans. **Fig. 4.** Pictogram showing cloves sorted by area and then color coded according to their bin placement. **Fig. 5.** Image showing unsorted pieces of quick oats. **Fig. 6.** Image showing an array of sorted pieces of quick oats. Note that many particles were not separated correctly because they were touching in the original image (Fig. 5).

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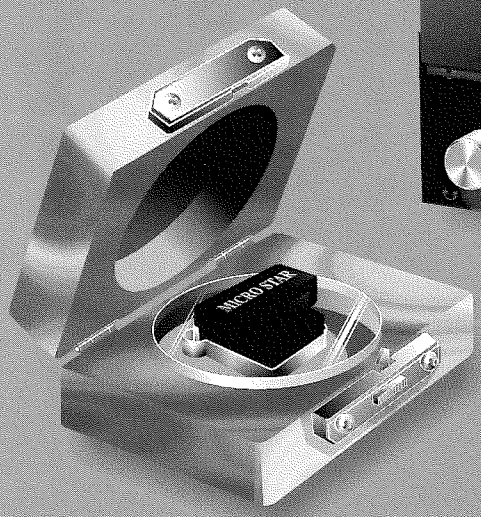

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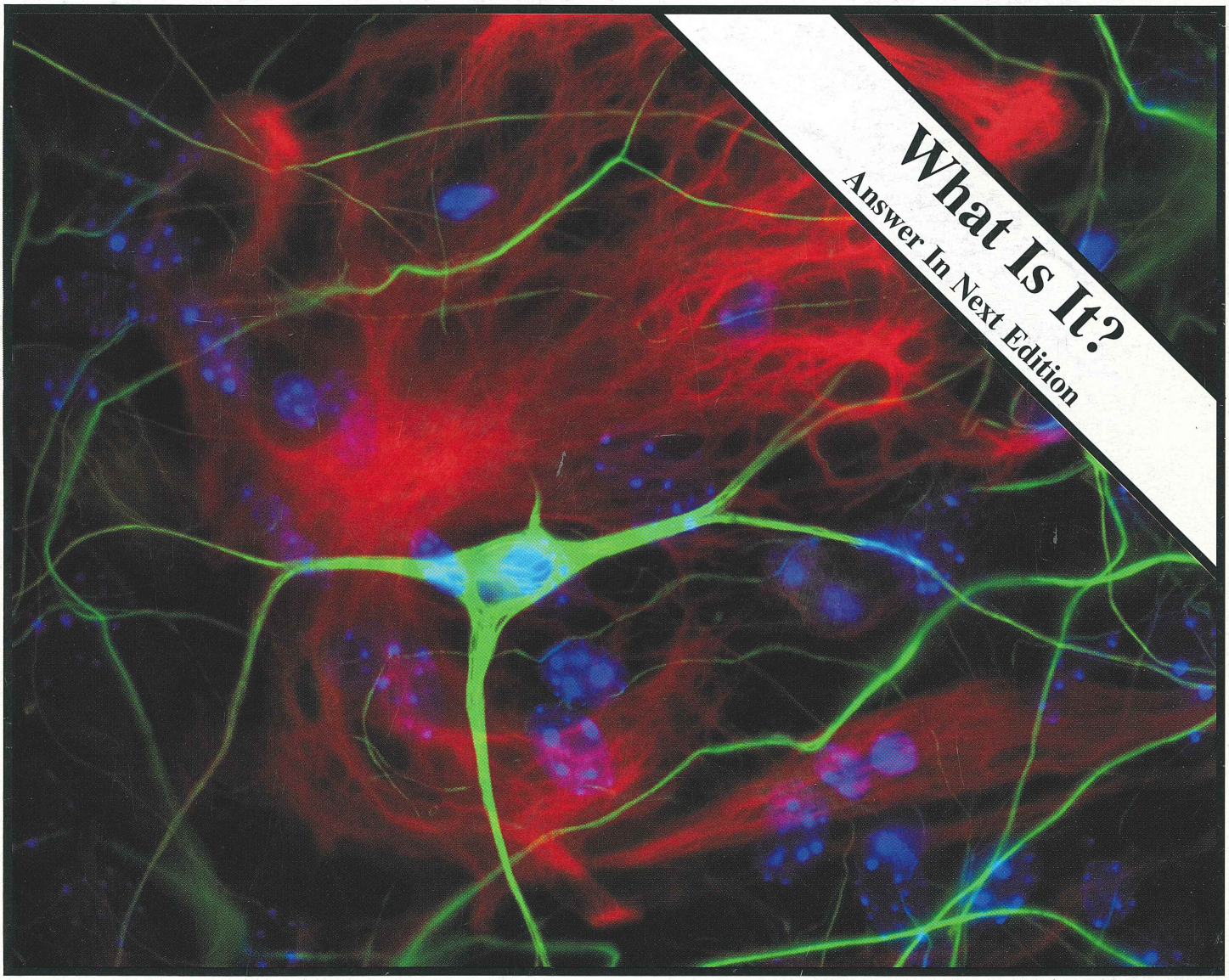

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