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# Automated Detection of Karnal Bunt Teliospores

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## ABSTRACT

Karnal bunt is a fungal disease which infects wheat and, when present in wheat crops, yields it unsatisfactory for human consumption. Due to the fact that Karnal bunt (KB) is difficult to detect in the field, samples are taken to laboratories where technicians use microscopes and methodically search for KB teliospores. AlliedSignal Federal Manufacturing & Technologies (FM&T), working with the Kansas Department of Agriculture, created a system which utilizes pattern recognition, feature extraction, and neural networks to prototype an automated detection system for identifying KB teliospores. System hardware consists of a biological compound microscope, motorized stage, CCD camera, frame grabber, and a PC. Integration of the system hardware with custom software comprises the machine vision system. Fundamental processing steps involve capturing an image from the slide, while concurrently processing the previous image. Features extracted from the acquired imagery are then processed by a neural network classifier which has been trained to recognize "spore-like" objects. Images with "spore-like" objects are reviewed by trained technicians. Benefits of this system include: (1) reduction of the overall cycle-time; (2) utilization of technicians for intelligent decision making (vs. manual searching); (3) a regulatory standard which is quantifiable and repeatable; (4) guaranteed 100% coverage of the cover slip; and (5) significantly enhanced detection accuracy.

**Keywords:** image processing, neural network, machine vision

## 1. INTRODUCTION

The United States (US) produces approximately one-third of the world's wheat, with the state of Kansas producing approximately one-third of the US total[1]. Consequently, Kansas has a tremendous stake in US wheat production. On March 8, 1996, the US Department of Agriculture (USDA) announced that Karnal bunt (KB) had been detected in several seed lots of durum wheat grown in southwestern Arizona[2]. Consequently, the USDA put the entire state of Arizona under a Karnal bunt quarantine, along with two counties in southeastern California, four counties in southwestern New Mexico, and two counties in west Texas[3]. Previously, the Kansas Department of Agriculture (KDA) had coordinated a USDA-funded regional KB survey between 1993 and 1995. Wheat samples collected from regional elevators were analyzed by the KDA for Karnal bunt and all were found to be negative. Following the USDA Karnal bunt quarantine, all 42 wheat growing states participated in a greatly expanded KB survey. There were two reasons for performing this survey. First, undetected pockets of Karnal bunt could potentially be identified and subsequently eradicated before spreading. Second, wheat exports were facilitated from areas where KB was not found. While Karnal bunt is not life threatening when it is consumed by humans, it does create a "fishy" taste, thereby significantly reducing its value by approximately 50 percent.

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Karnal bunt is a disease which infects common wheat, durum wheat (used for pasta), and triticale (a hybrid of wheat and rye). It is caused by a fungus called Tilletia indica. In the springtime, infections penetrate individual florets, enter the embryo end of the kernel, and proceed along the crease. Infected portions of the kernel are marked by masses of dark, powdery, fishy smelling, fungal teliospores. Infection can vary from a trace to the entire kernel. Infected kernels are weak and easily break open to release the Karnal bunt teliospores.

Due to the fact that Karnal bunt is difficult to detect in the field, samples are taken to laboratories. Here the grain is washed, centrifuged, and sifted through multiple filters. Fifty gram grain samples are agitated for five minutes in a detergent solution, freeing any fungal spores. The wash solution, which now contains the freed spores, is passed through a 52 micron sieve and a then a 20 micron sieve. Target spores are caught on the 20 micron sieve. The screenings are then centrifuged and pelleted. The pellet is then re-suspended in Shear's media. Slides are made from the suspension, and then sealed and labeled. These slides are then examined for KB teliospores. Technicians sit at microscopes and methodically and laboriously search for Karnal bunt teliospores. Detection of one or more teliospores is considered a positive test. This detection process is very labor intensive; operators are allowed to work no more that six hours per day, due to fatigue. Because this fatigue factor could cause technicians to fail to identify a KB teliospore, every slide is scanned twice.

Recognizing KB scanning as a manually intensive problem, the KDA, with the assistance of AlliedSignal Federal Manufacturing & Technologies (FM&T), created a system which utilizes pattern recognition, feature extraction, and neural networks to create an automated scanning system for detecting KB teliospores. The time from the inception of this project to the delivered system was two months, allowing the KDA to use this system for the next wheat harvest. Using this system, Karnal bunt spores are distinguished from clutter and other types of teliospores present on the slide. Images which contain "spore-like" objects are presented to a trained technician for final verification. This automated system changes the technician's role from the one who meticulously scans slides to the one who verifies the results of the scanning process.

A Karnal bunt teliospore is a spherical object whose cross section is circular to slightly elliptical in shape. A mature teliospore ranges in size from approximately 22 to 47 microns. They are dark in color, with a transparent sheath along the perimeter. Immature teliospores are smaller in size and relatively transparent. If an immature teliospore is on a slide, it is assumed that there will be mature teliospore present on the slide also[4]. Therefore, the scope of this work includes searching for mature teliospore only. Examples of scanned KB spores are shown in Figure 1.

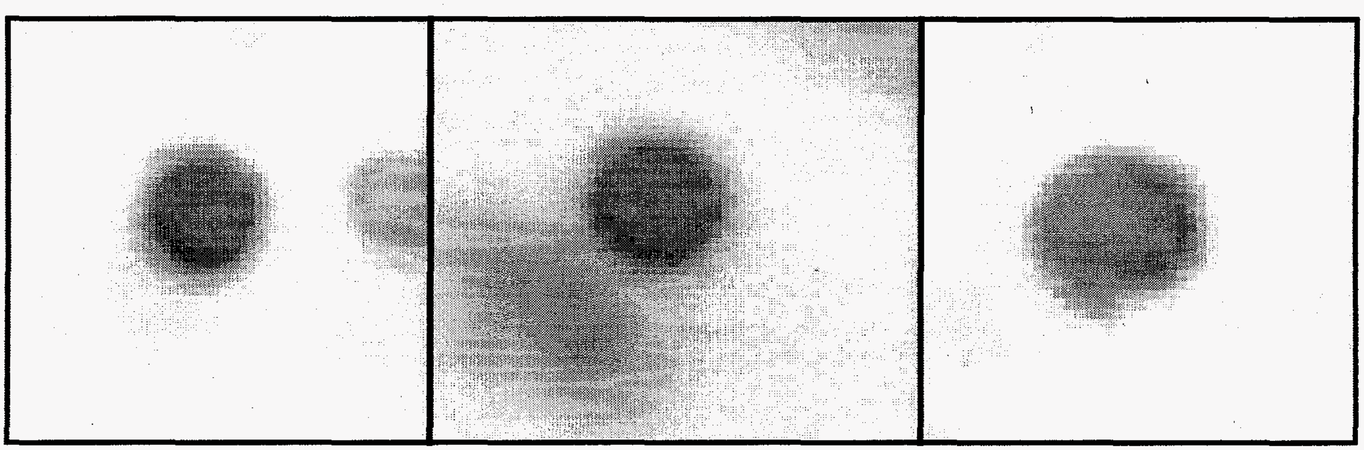


**Figure 1.** Three Karnal bunt teliospores as captured via the scanning system.

## 2. SYSTEM DESCRIPTION

The system hardware consists of a biological compound microscope, CCD video camera, programmable motorized microscope stage, video monitor, and a PC with a video capture card. System integration of these parts, along with custom software, makes up the machine vision system. This system is illustrated in Figure 2. The fundamental processing steps involve capturing an image from the slide, while concurrently processing the previous image. Image segmentation and feature extraction techniques are applied to each acquired image in order to isolate potential spores and provide a set of descriptive characteristics, or features, for each detected object. The extracted features are then processed by a back propagation neural network which has been trained to recognize "spore-like" objects. A "confidence" value is also produced for each detected object.

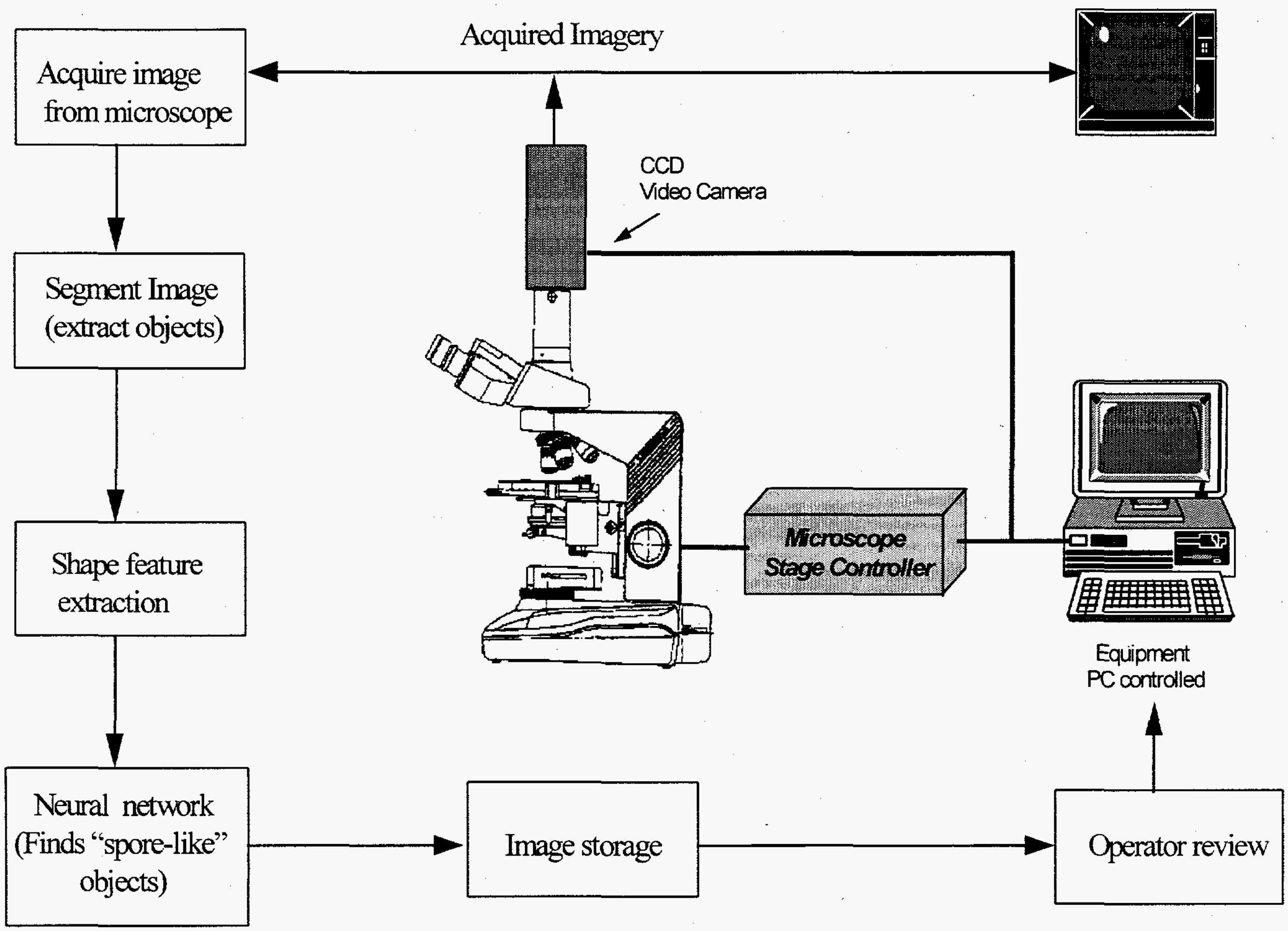



**Figure 2.** Hardware and data flow of automated scanning system.

Images which contain "spore-like" objects and have a high confidence value are saved for verification by a trained technician after the entire slide has been scanned. This technique changes the role of the technician, or operator, from the one scanning for the spore to the one who verifies the automated findings of the system. After all images and extracted objects have been processed, the operator is shown the images which have the highest confidence values (i.e., those which contain objects which are most "spore-like") of the slide. The operator then makes the final identification of the Karnal bunt teliospore.

## 2.1 Hardware

System hardware consists of commercial off-the-shelf products. A black and white CCD video camera (Sony model SSC-M370 c-mount) was mounted to a Nikon Labophot-2® microscope for image acquisition. The Nikon CF® optics provides a sharp, bright image for data capture. A Nikon Biostation Cytology System is used for slide positioning. The Biostation includes a computer-driven motorized XY stage and a manual Z focus system. The XY system is used for automatic scanning of individual slides. As the slide is positioned to acquire an image, overlap in both directions is used to assure that no teliospores are missed due to the fact that the spore lies partially on one image and partially on another, or worst case, that a teliospore lies amid four adjacent images. When images are presented for review, the XY system relocates the slide such that the operator may refer back to the monitor *or* microscope for the review process. During review, the operator will often change microscope objectives to better examine the spore in question. An Alacron video frame grabber card is installed in a 166 Pentium PC for acquisition of a 480 by 640 pixel image. The acquired image is passed to the image processing and feature extraction software for processing.

## 2.2 Software

The Karnal bunt scanning software (written in LabView and DOS) presents a user friendly graphical interface in a Windows NT environment. The user is stepped through a process of adjusting the microscope light, placing the slide on the stage, and defining the area of interest on the slide. Typically this area would be the entire cover slip. The software then proceeds to scan the slide, an image at a time, in a predetermined path. Each image is passed to the image processing routines (written in C) and processing begins, while at the same time the slide is repositioned to the next image location, and the next image is scanned. This process is repeated until the entire area of interest is scanned and processed. Note that the slide positioning and image acquisition is taking place concurrently with the processing of the previous image (except, of course, for the first image and final processing). By executing these processes at the same time, the total system execution time is essentially cut in half.

At the heart of this automated system is a pattern recognition routine which has been trained to detect the Karnal bunt teliospore. The initial stage applies a customized image segmentation procedure to extract objects (or blobs) from the image. This customized process includes: inverting the image, performing a histogram equalization (to enhance the light and dark contrast between given pixel values), and binarizing the image[5]. A "block" (or integrated energy) algorithm is then applied to the binarized image. This block algorithm is used to identify regions of interest within the image which have a higher likelihood of containing a teliospore, while eliminating from further processing those areas of lower likelihood. If no regions within the image are identified with high likelihood, the routine proceeds to analyze the next image. If at least one region of high likelihood is identified, a correlation (or template) algorithm is applied to the identified region. A low correlation value dictates that the object is not enough like a spore to perform any more processing, and thus the object is discarded. A mid to high correlation value indicates that the object is "spore-like" enough to dictate further processing. When an object is determined to be "spore-like, a number of morphological features are calculated and used to uniquely describe the suspected spore. Features which are calculated include: area, perimeter, circularity, and fractal dimension. A flowchart of this procedure is shown in Figure 3.

The extracted features for each suspected spore are used as input to a back propagation neural network[6] (written in C). This neural network has been previously trained on images which contain known Karnal bunt spores. A neural network was used for this recognition task since Karnal bunt teliospores can exhibit a considerable range in characteristics (shape, size, density, etc.) and because there was a sufficiently large amount of imagery available for training the neural network. In addition, the acquired images often contain many objects which are *not* spores (clutter) and which the neural network had to be trained to reject. Such objects included dirt, air bubbles, metal filings, and other debris in the washed sample. Figure 4 illustrates objects which are spore-like, non spore-like, and an actual Karnal bunt teliospore.

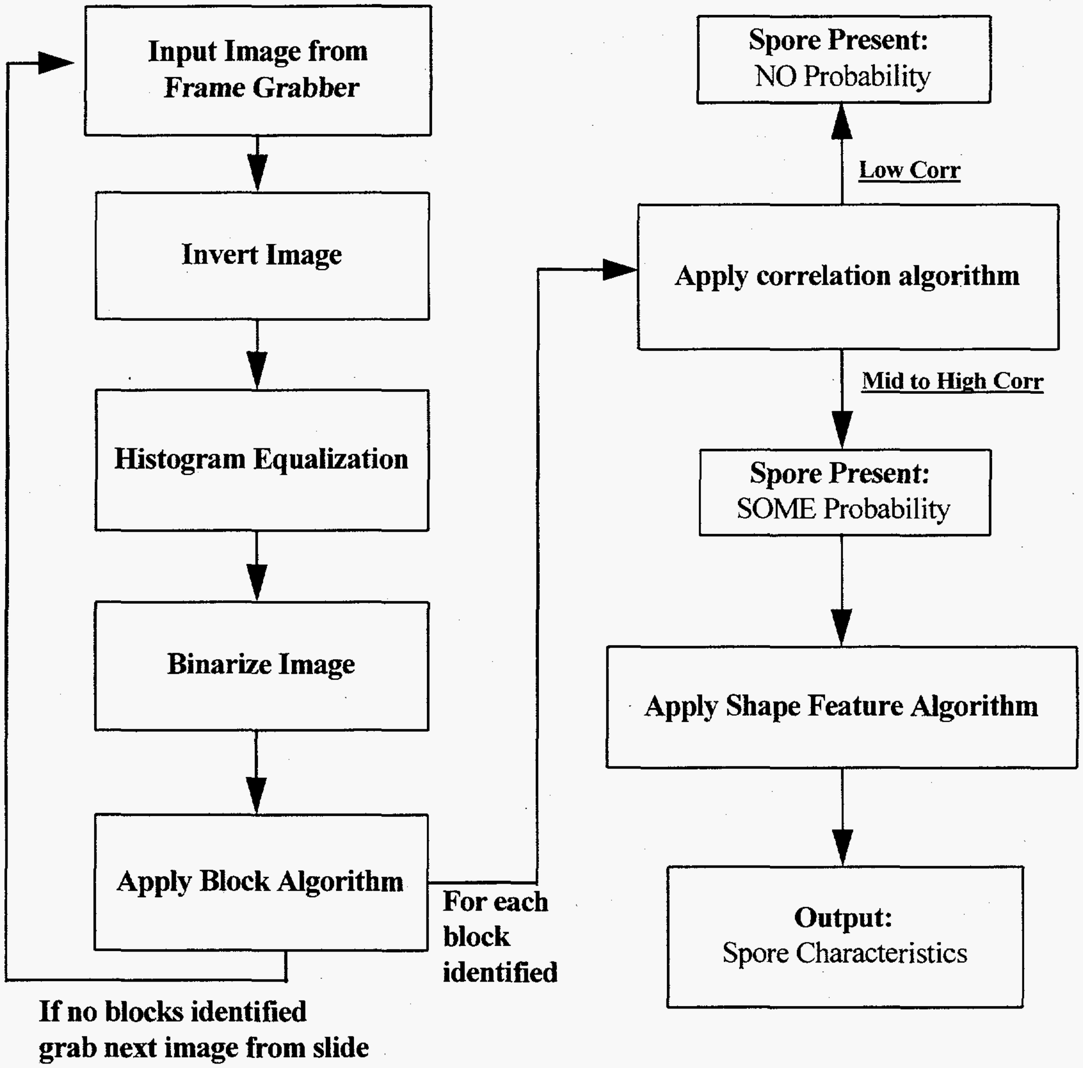



**Figure 3.** *Flow chart of feature extraction routine.*

## 3. RESULTS AND CONCLUSIONS

Several slides were provided by the KDA to perform a rigorous test and validation of the scanning system. These slides were known to have Karnal bunt teliospores on them. While some slides contained wheat samples which had been washed, centrifuged, and filtered, other slides contained samples which had not been filtered (thus creating a cluttered slide). Because the centrifuging and filtering requires time (and money), it was of interest to see if this step could be eliminated.

The KDA sample slides were labeled B-1, B-2, C-4, and E-3. Of these slides, B-1, B-2, and E-3 are considered "clean" slides, meaning that they had been thoroughly washed and filtered. Slide C-4 had not been filtered and is considered a cluttered slide. To present the difference between a cluttered and clean slide, a sample image from a clean slide as compared to a sample image from a cluttered slide is shown in Figure 5. The impact that a cluttered image has over a clean image is that it will require more processing time. This is due to the fact that more objects (potentially "spore-like") will initially be found on a cluttered slide, which will require more overall processing to determine if each object is actually "spore-like" or not.

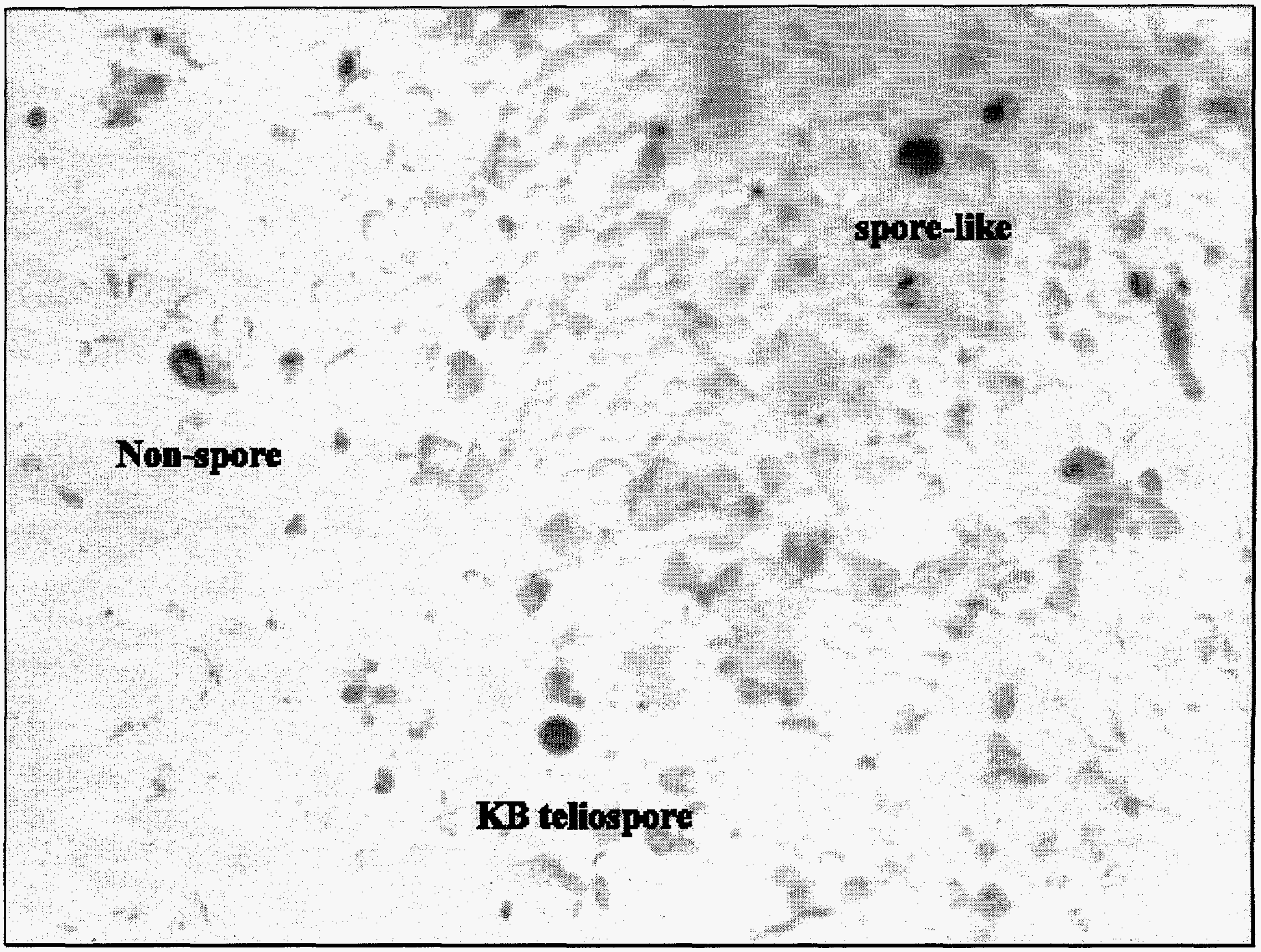
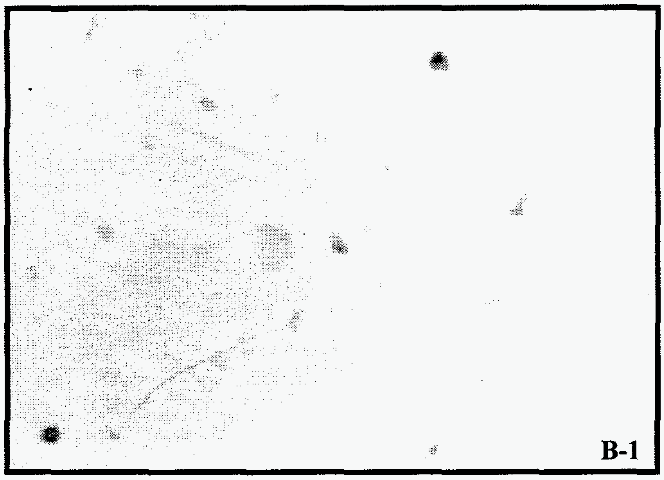



**Figure 4.** Example of acquired image with spore-like and non spore-like objects.

Results from running the four slides through the scanning system are given in Table 1. The first column gives the sample slide label, followed by the number of spores on the slide and the number of those detected in the scanning procedure. Note that the column heading # **Detected** does *not* refer to the number of KB teliospores detected; rather, it refers to the number of "spore-like" objects detected during the scan. The final identification of a KB teliospore is performed by a trained operator. As shown in the second and third columns, all spores were detected on the clean slides, and all but five (93.5%) were detected on the cluttered slide. This is acceptable for the KB scanning system. The case will not occur in which a single, lone spore is present on a slide; if there is one spore, there will be many[4]. The goal of this scanning system is to detect if any "spore-like" objects exist on the slide, not to compute the actual number.

The fourth column of Table 1 refers to the number of objects found from the block algorithm, followed by the final number of "spore-like" objects determined by the neural network. The images containing these "spore-like" objects are presented to the reviewer with the highest ranking confidence values presented first. Recall that only the existence of a single spore is necessary on a slide to determine that the wheat is infected with Karnal bunt. The final two columns of Table 1 indicate processing time for both the entire slide and the average time per image. These times were computed on a PC with 33MHz CPU speed. The delivered system contained a 166 Pentium PC. Note that the clean images required less processing time, in general, than the cluttered images.



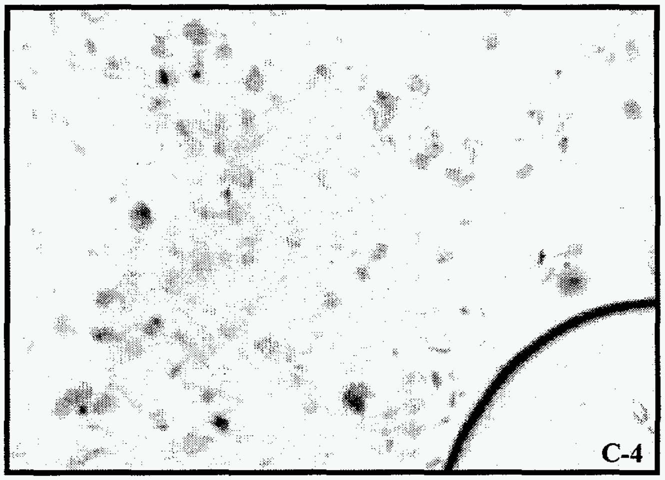




**Figure 5.** Sample images from a clean slide (B-1) and a dirty slide (C-4).

**Table 1**. Performance of scanning system on four sample slides.

| Slide | # of Spores | # Detected | Initial Hits | Objects to Review | Time/Slide | Time/Image |
|---|---|---|---|---|---|---|
| **B-1** | 10 | 10 | 559 | 124 | 19.3 min. | 2.07 sec. |
| **B-2** | 13 | 13 | 543 | 127 | 19.6 min. | 2.10 sec. |
| **C-4** | 62 | 58 | 2397 | 294 | 26.8 min. | 2.87 sec. |
| **E-3** | 15 | 15 | 2794 | 272 | 29.3 min. | 3.03 sec. |

While the automated Karnal bunt scanning system has performed well for detecting "spore-like" objects, the system does have limitations. As seen by the results for sample slide C-4, spores can be missed. Typically this is caused by a situation when a teliospore is adjacent to, and touching, other dark objects such as clutter. Additional image segmentation techniques could address this. There are two other situations when a Karnal bunt teliospore will not be detected by the scanning system: 1) flattened spores and 2) broken spores. A flattened spore typically occurs when the cover slip on the slide is pressed down with enough force to squash the spherical teliospore. This mishandling results in a change in the spore's cross-sectional morphology. Typically the shell will partially split, changing some (but not all) of the characteristics, or features, associated with the object. Features which will change include the perimeter and correlation (template) values. Special software could be added to search for flattened spores. However, for this application, this was not deemed necessary, as proper handling of the slide will prevent this situation from occurring. While a flattened spore is a single, unsegmented object, a broken spore consists of many discrete pieces. Broken spores can be of any shape or size (obviously, smaller than the size of a teliospore). To scan for broken teliospores is beyond the scope of this project.

## 4. BENEFITS

There are many benefits that the Karnal bunt automated scanning system provides over the previously used manual process. First and foremost, the per slide scanning speed has been reduced from approximately 40 minutes (for a trained technician) to less than 10 minutes (automated process). This allows for an average daily slide output of 100 per day, compared to 10 per day for a single microscope using the manual process. Thus, the scanning cost per slide is considerably reduced compared to the manual method. Moreover, 100 percent slide coverage is guaranteed, precluding the need for a second review of each slide (as is routinely performed in the manual process). A summary of benefits provided by the automated scanning system include:

- Reduction of the overall cycle time of the scanning process.
- Utilization of technicians for intelligent review and decision making rather than manual scanning.
- A regulatory standard which is quantifiable and repeatable.
- Guaranteed 100 percent coverage of the cover slip.
- Significantly enhanced detection accuracy.

## 5. ACKNOWLEDGMENTS

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