Texture Analysis for Nematode Genera Classification

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ABSTRACT
Nematodes are the most diverse animals and the most numerous multi-cellular organisms on earth. They exist in almost every ecological niche and vary in size from microscopic round worms to large classes such as Cestoda, which can grow to over 30 meters. Not only are nematodes greatly abundant, but they have significant impacts on human life. Nearly two billion people worldwide are infected by a single nematode genus and billions of dollars of yearly agricultural damage can be attributed to just a handful of nematode species. Because these affects originate from a small subset of the phylum, classification of nematodes is necessary to assist disease prevention and eradication. Genetically based pesticides rely on accurate identification and monitoring of nematode populations. Classification of a single nematode by field experts requires several days, an automated system would improve the accuracy, throughput, and efficiency of this process. This paper focuses on classifying microscopic nematodes. This presents additional challenges in classification due to the size, lack of visual features, and great abundance of the subjects. Microscopic nematodes have simple, transparent bodies. A texture based analysis is conducted towards classification of a sample's genus due to their structure. Nematode images are attained using video capture and editing microscopy (VCE) techniques, resulting in a series of multifocal images per nematode sample. By applying texture based methods on multifocal image stacks of nematodes, an accurate image based classifier is constructed.

Categories and Subject Descriptors
H.2.8 [**Database Management**]: Database Applications – *Data mining and image databases.*

General Terms
Measurement, Performance, Design, Experimentation

Keywords
Image Similarity, Texture Analysis, Nematode Classification

1. INTRODUCTION
Nematodes are the most numerous animals on earth. In a handful of dirt, there can exists hundreds of thousands of nematodes. Fortunately, not all nematodes pose a threat to humans. Because of the large influence a relatively small subset of the phylum has on humans, there is a necessity for identifying adverse nematodes. Nearly 2 billion humans are infected worldwide by just a single nematode genus, Ascaris[1]. Nematodes are a common pest to agriculture production. Only a handful of the phylum are to blame for the estimated $157 billion in yearly damage around the world[2]. The size, appearance, and abundance of microscopic nematodes provide difficulties to image based classification systems. Due to their simple structure and lack of visual characteristics, a texture based analysis of their images is conducted towards genus classification. In section 2, I cover work related to micro organism and texture classification. Section 3 describes the physical structure of microscopic nematodes and the difficulties in their classification. Section 4 presents the multifocal image stacks of nematodes which were provided by [3]. Section 5 illustrates the feature extraction algorithm used for the nematode image stacks. Section 6 covers classification methods based on the extracted texture features. Section 7 presents experimentation and results of my method on the dataset with a discussion of the results in section 8. The paper then concludes in section 10.

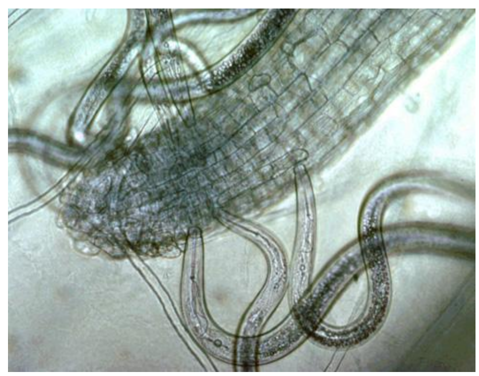

Figure 1 - Microscopic nematodes feeding on plant cells

2. RELATED WORK
Classification has been done on similar animals such as diatoms [3][4], which are simple unicellular microscopic organisms. Similarly to nematodes, diatoms are small, plentiful, and have similar visual characteristics but exhibit symmetricity in their shapes and internal patterns. Because of their symmetric features, many image based classifiers depends on edge detection and symmetrical analysis. These methods could not be applied to nematode classification and texture based approaches for diatoms are scare and are given inadequate effort.

Texture analysis has been conducted in many applications, one of which is object detection. The author of [5] utilize a texture based detection method which features some degree of scale, rotation, and illumination invariance. Unfortunately, nematode images are highly exhibit noise due to their transparency and lack of physical structures. To accommodate for this I utilize a less strict feature matching technique and exploit the multifocal property of the dataset.

3. NEMATODES

Microscopic nematodes are transparent and most of what can be seen is digested food and fat cells. Due to this abundance of uncharacteristic features a visual classification is difficult, requiring 3-5 days of professional analysis. They have a simple tube like structure, described by experts**Error! Reference source not found.** simply as, "a tube in a tube". As segmented worms, they grow by replicating their midsection. This growth processes leaves little characteristic features in the midsection, leaving only the head and tail for classification purposes. The head of animal contains the most distinct features (i.e. teeth. lips, throat, etc.) and is therefore used in this paper's classification.

4. DATA REPRESENTATION

The nematode samples are gathered by Dr. Paul De Ley et al.**Error! Reference source not found.**. The microscope is an Olympus BX51 with transmitted light arm, lamphouse, 8-position universal condenser, mechanical stage, 7-position DIC nosepiece and trinocular observation tube with 10x eyepieces. The video camera is an Olympus OLY-200 1/3" single CCD color video camera. The VCE process begins by positioning the animal and initializing the focus of the microscope to view the animals surface. Video recording begins and the focus of the microscope is sent through the specimen. Figure 2 displays the frames of the supplied data as an image volume. The video is then post processed for crop adjustments and redundant frame removal. Though the process is standardized, it is prone to human error and different nematodes may vary in image count, solution color, and subject to background ratio. Because of the VCE system, registration of the images is not necessary. For color invariance from the solution type, each image is converted to gray scale.

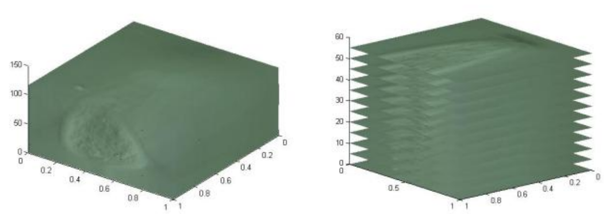

Figure 2 - Focal images of a single nematode stacked

5. FEATURE EXTRACTION

5.1 Local Gradient Analysis

For local gradient analysis, a window size, w, and orientation bin count, $binCount$, are defined. A $w \times w$ grid is arranged over every image. Figure 3 illustrates a gray scaled image of local image grid. For each pixel in the grid, the local intensity difference on the horizontal and vertical axis are compared and an orientation and magnitude is calculated by comparing the surrounding pixel intensities.

$$pMagnitude = \sqrt{(pRight - pLeft)^2 + (pUp - pDown)^2}$$

$$pOrientation = arctan(pMagnitude)$$

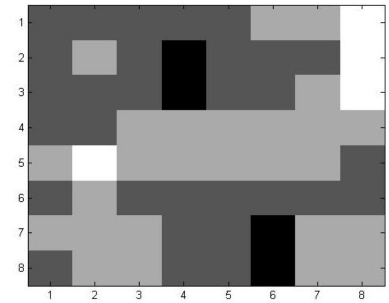

Figure 3 - Local grid with W = 8

Pixels on the edges of the image are extended to reduce large gradient changes found in other techniques, such as zero padding. The orientation space is binned into $binCount$ degree segments. Each bin has an associated reference angle, which is the degree value at the bin's midpoint. Aggregating the magnitude and orientations of each pixel creates a *local gradient descriptor* for each grid. The corresponding bin which holds $pOrientation$ is incremented by $pMagnitude \times pWeight$. The value of $pWeight$ is inversely proportional to the distance of $pOrientation$ from its bin's reference angle.

$$pWeight = 1 - \frac{|\,referenceAngle - pOrientation\,| \times binCount}{\pi}$$

Weighing the orientation angles during binning provides a better approximation of the most dominant orientation on the grid.

Because the information from natural textures is mostly concentrated off the diagonals[7], the bins corresponding to diagonal orientations can be removed from local gradient descriptors that utilize a $binCount = 8$. This reduction leaves bins for the directions up, down, left, and right.

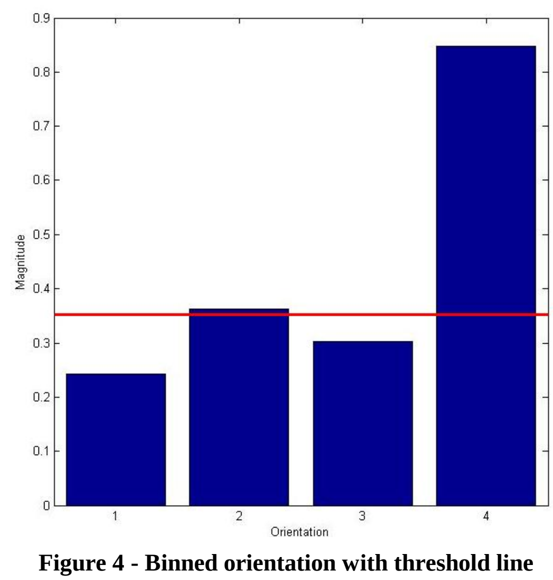

Figure 4 - Binned orientation with threshold line

5.2 Illumination Invariance

For global illumination invariance the orientation histogram is then normalized to 1. The normalization of the values allows for each grid to be compared relative to their own pixel intensities. For local illumination invariance the values of each bin are then thresholded to $binThreshold$. If there is a significant difference in local intensities (i.e. cause by an edge with a shadow) peaks will form after the histogram is normalized. To counteract this the values of the bins that are over the threshold are set to $binThreshold$. In effect, this process reduces the contrast of the image relative to a single grid. Shaded areas will become brightened and near white out segments will be toned down. Areas that do not exhibit such features will remain unchanged since there should not be much change for windows with small values of W. Figure 4 displays the non diagonal bins of a local gradient descriptor and along with a thresholding line.

5.3 Rotation Invariance

To remove dependence on the orientation of the nematode, each local gradient descriptor is aligned to one another, similar to the rotation invariance in [5], by rotating them according to their most dominant orientation. Each local gradient descriptor vector is circularly rotated to maximize

$$\sum_{i=1}^{binCount} binValue(i) \times i$$

Maximizing on this equation results with local gradient descriptors which tend to have larger values toward the end.

5.4 Background Removal and Clustering

Once local grid descriptors have been calculated for all images in the training set they are clustered into k groups through kmeans clustering. The centroids of the clusters are then used to assign each grid to k descriptor bins. In the data domain of nematode images the background texture is consistent and abundant. To exploit this observation the largest $\frac{k}{4}$ bins, having been empirically found to contain the majority of the background grids, are discarded. Open and closure morphological operations are then performed to smooth the background classification results. The remaining grids are then re-clustered and the *descriptor centroids* are saved for classification of new samples. Figure 5 displays a single focal plane with descriptor centroids and background grid assignments annotated. The morphological operations can cause some inclusion of the background, but overall allows the descriptors to focus on the nematode textures.

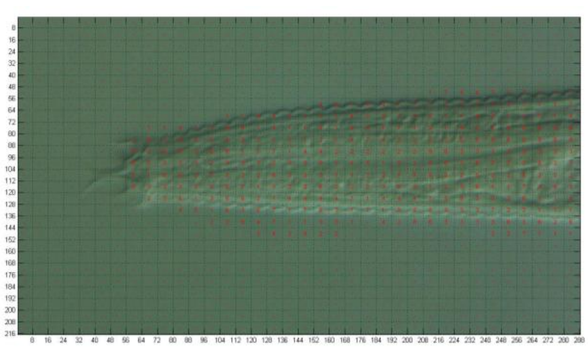

Figure 5 - Focal slide annotated with their assigned descriptor bins.

With each grid assigned a descriptor bin, a focal slide from a nematode can now be represented by its *descriptor histogram*. Figure 6 displays the descriptor histogram of 24 individual nematodes from 4 different genera. Each line represents the descriptor histogram of an individual's center focal plane image while the color of the line signifies genus membership. The similarity of individuals within the same genus can be clearly observed along with the differences between genera.

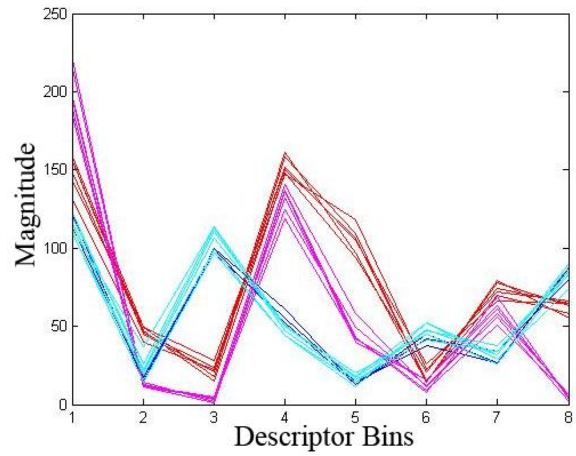

Figure 6 - Descriptor bin histogram of 4 genera with 6 individuals each

6. CLASSIFICATION

6.1 Training

To train the classifier, the descriptor centroids across all images in the training set are calculated. The descriptor histograms for each image are then gathered and associated to their genus class and source individual. A second method which trains on a subset of the dataset is described in section 6.4.

6.2 Descriptor Euclidean Distance

Queries are submitted to the classifier as a stack of focal plane images from a single individual. The descriptor histogram for each image in the query is calculated and its nearest neighbor in each genus is found. The distance measure used is simply Euclidean distance between the image descriptor vectors.

6.3 Weighted Slides

Aggregation of the distances for each slide and for each genus is then conducted. Some slides in the multifocal image stack prove to be more useful for classification than others. For each image in the query individual a slide weight, $slideWeight$, is calculated.

There are two variants used:

1) *Normalized distribution of weights*. Slides near the center of the stack are given more weight than those at the beginning or end. Slides at the center of the image are focused on the internals of the nematode (mainly the digestive tract and inner mouth), where most of the information used by experts is located while the outer areas contain mostly fat cells. The center of the animal also provides the largest cross section for visual analysis. This weight distribution therefore classifies nematodes more strongly with the features found at the center focal planes.

2) *Standard deviation based weights.* The standard deviation of each query image is calculated and are then normalized to 1. Figure 7 displays the standard deviations of each slide in a query stack. Focal planes on the above and below the nematode will

mostly consist of background texture. Due to this there will be a low amount of change in the intensities of the image at these images. Images which capture organs and other visually interesting features will exhibit higher variance from the edges of the structures. As the focus moves past the center of the image, noise from out of focus objects in above planes causes the usefulness of the slides to decrease. This will cause a blurring of the image and reduce the deviation in pixel intensities. If a nematode has a highly descriptive surface, then there will be more deviation on the upper slides.

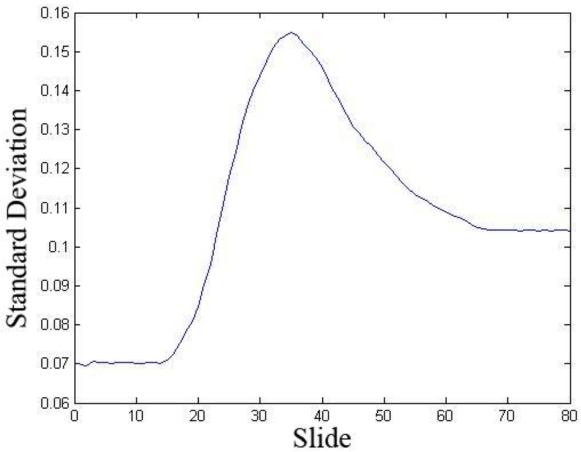

Figure 7 - Standard deviation calculated from a query stack

The nearest neighbor distance from each genus for each slide is then modified by it's $slideWeight$, which is then summed to attain a distance from each genus. Membership for the query individual is given to the genus with minimum distance.

6.4 Representative Selection

Another classification method with a multifocal image stack is to select representative images from each individual's image stack. The standard deviations of each image within a stack are again calculated and the L images with the largest deviations are used for training and classification.

7. EXPERIMENTATION

7.1 Data

For testing and training, 5 genera are selected for classification. In each genera, image stacks for 10 individual nematodes are analyzed. Each individual has, on average, 160 images in it's multifocal image stack. Examples from each genera are given in Figure 8. Figure 9 displays parameters given fixed values found beneficial through empirical results. Parameter L is set to 1 for simplicity. Leave one out cross validation is used to measure the accuracies of each experiment. The class representation is kept equal by leaving out one individual from each genus and then testing with the excluded set. Multiple combinations of excluded sets are tested. Base accuracy, through random guess, in this data set is 20%.

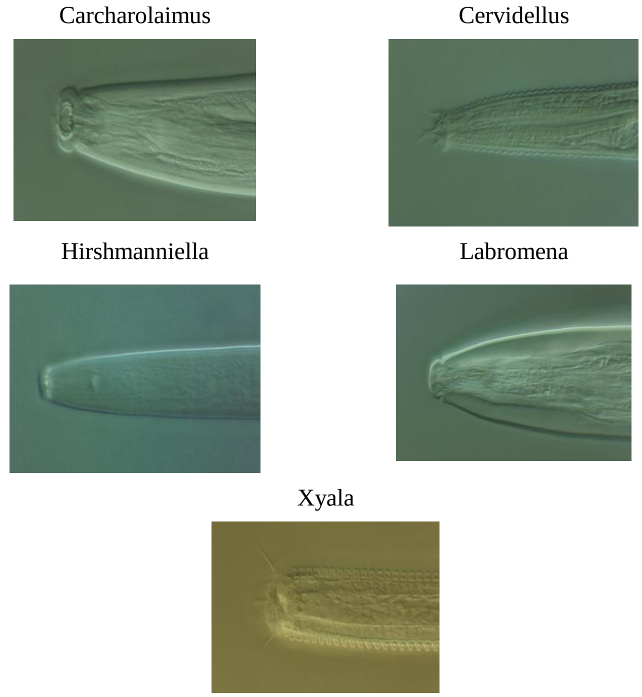

Figure 8 - Examples from each genus taken at the center focal point

Local grid dimension w	8
Local illumination threshold $binThreshold$	.35
Descriptor bin count k	8
Representative images count L	1

Figure 9 - Fixed parameters

7.2 Experiment Scenarios

The entire data set is test with $binCount$ values of 4 and 8 and with diagonal bins intact. The normal distribution is used to weigh each slide during classification. Both values of $binCount$ yield an accuracy of 86% with differing predictions between the genera Carcharolaimus and Cervidellus.

Normally distributed weights were tested against standard deviation weights with $binCount = 8$, both featuring removal of diagonal orientations. The normal weights provide an accuracy of 74% while the standard deviation weights yielded 86% accuracy.

Representative selection is then used for training and testing. With standard deviation weights and $binCount = 8$, an accuracy of 80% is found.

Best results were found with $binCount = 8$, retaining diagonal bins, and forgoing local illumination invariance. This setup yielded an accuracy of 90%.

8. DISCUSSION

Modifying the orientation count showed no improvement in testing accuracy. This is due to the standardized VCE method for data gathering which provides a consistent orientation to the images. Because of this stability, segmenting the orientation space into small pieces (or aggregating into fewer bins) does not greatly change the region in orientation space that a local grid may be binned to. This also has an effect on the diagonal removal method where, unlike the results found in [7], removal of diagonal measurements reduced the accuracy of the classifier because changes to *binCount* mainly modify the length of the descriptor vectors while retaining the same trends in its shape and discarding bins from the descriptor could remove relevant information for classification. The *binCount* and diagonal removal settings found through experimentation could then be over fitting to this standard of data retrieval and may be useful once the dataset grows to include images from other image providers.

Extracting the descriptors for about 8000 high resolution images takes about 5 hours on a AMD Phenom 2.3 GHz quad core with 8 GB of memory. Cross validation then takes about 45 minutes. Utilizing only the representative image the entire process reduces to about 5 minutes. Although the resulting accuracy when compared to a process which involves all images is lower, it is still a 60% increase over the base accuracy and at a fraction of the processing time.

The best results were discovered through disabling the local illumination invariance in the local grid analysis phase. This is again a result of the standardized image collection method. Since the researchers attempt to keep illumination across images constant, modifications based on its invariance are likely to remove important data. Again, this result may be over fitting to this standard and could be helpful as the dataset expands.

9. FUTURE WORK

Construction of method with a lower amount of parameters is necessary to avoid over fitting and to increase ease of implementation and applicability to non nematode datasets. A multi-resolution analysis of the data could improve on the results found by classifying with texture features found at different scales of the nematode images. Preprocessing filters could also accentuate textural features that may improve classification accuracy.

10. CONCLUSION

By using texture analysis methods on multifocal images of microscopic nematodes I was able to build classifiers with accuracies well over base accuracy. These classifier featured invariances in color, global illumination, local illumination, and rotation. Though the process involves a large set of parameters, it also features the ability to be adjusted for a currently growing dataset of nematode images. By tuning these parameters a classifier can be tailored to fit with a large assortment of nematodes which vary in both internal and external characteristics. Though the all inclusive data method requires time to cross validate, a single classification can be preformed in seconds. The entire process is still faster than one performed by a field expert. Using this classification tool experts can reduce their workload by focusing on nematodes poorly classified by the system.

11. REFERENCES

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