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Shape-Based Classification of Environmental Microorganisms

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***Abstract*—Occurrence of certain environmental microorganisms and their species is a very informative indicator to evaluate environmental quality. Unfortunately, their manual recognition in microbiological laboratories is very time-consuming and expensive. Therefore, we work on an automatic method for shape-based classification of EMs in microscopic images. First, we segment the microorganisms from the background. Second, we describe their shapes by discriminative feature vectors. Third, we perform the EM classification using Support Vector Machines. The most important scientific contribution of this paper, in comparison to the state-of-the-art and to our previous publications in this field, is the introduction of a completely new and very robust 2D feature descriptor for EM shapes. Experimental results certify the effectiveness and practicability of our automatic EM classification system emphasising the benefits achieved with the new shape descriptor proposed in this work.**

I. Introduction

Environmental Microorganisms (EMs) are microscopic beings living in the natural (rivers, seas, forests, mountains, etc.) and artificial (fields, gardens, fish ponds, aeration tanks, etc.) surroundings. Their classification is a very important indicator for biological treatment processes and environmental quality evaluations. Unfortunately, it is very difficult to distinguish thousands of EMs from each other. Traditionally, they are recognised manually in environmental laboratories either by observing their shapes under a microscope (the morphological method [1]) or using molecular biology techniques. The morphological approach is much cheaper, but even very experienced operators are unable to distinguish thousands of EMs without referring to literature. The molecular technique distinguishes EMs by Deoxyribonucleic Acid (DNA) or Ribonucleic Acid (RNA) [2], [3] and is very accurate, but it is slow and expensive.

In order to overcome problems of these two methods, we have developed a practical and efficient system, in which microscopic images are automatically analysed to perform the EM classification. Our methodology simulates the morphological approach exploiting and modelling shape properties of EMs. First, the system conducts image segmentation to obtain EM shapes. Then, features characterising the shape of each EM are extracted from these segmented images. Afterwards, the class of the EM in an image is determined by a classifier based on its shape features. Finally, the result is feedbacked to the user.

The main contribution of this paper, in comparison to the state-of-the-art and to our previous publications in this area [4], [5], is a completely new and robust feature extraction technique picking the discriminative properties needed for EM classification in a very robust way. One has to mention here that feature extraction is an absolutely critical step in shape-based object classification. As can be seen in Section V, the usage of the new shape descriptor led to much better classification results.

The rest of the paper is structured as follows. Section II discusses related work in the area of computer-based microorganism analysis towards EM classification. Section III presents in detail the new shape descriptor introduced in this work. Section IV reports shortly on the classification method we have used. The effectiveness of the new feature extraction method is quantitatively validated with experiments in Section V. Finally, Section VI closes the paper with conclusions and insights into our future research plans in this area.

II. Related Work

In this section, we first describe existing approaches related directly to microorganism analysis (Section II-A). Second, we present available image analysis methods and select the ones that are suitable for our EM classification task (Section II-B).

A. *Classification of Microorganisms*

To the best of our knowledge, apart from our previous work [4], [5], there are no automated computer-based approaches towards EM classification based on the morphological strategy. However, there are some related classification methods for other types of microorganisms. We explain the novelty of our work by referring to TABLE I, which shows what types of microorganisms are addressed by the state-of-the-art systems.

Types of Microorganisms	Approaches
Environmental Microorganisms (EMs)	Our Previous Work [4], [5]
Medical Microorganisms (MMs)	[6], [7]
Water-borne Microorganisms (WMs)	[8], [9], [10], [11], [12]

TABLE I: Existing approaches applied for automated analysis of different types of microorganisms.

Medical Microorganisms (MMs) shown in the third row of TABLE I are investigated for prevention, diagnosis, and treatment of infectious diseases. For example, Rulaningtyas et al. present in [6] an approach for automatic classification of

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tuberculosis bacteria. For this, the authors use seven geometrical characteristics and neural networks for classification. Yeom et al. have developed a system for real-time 3–D sensing, visualisation, and recognition of MMs [7]. They extract features by Gabor-based wavelets and performed MM classification by automated training vector selection.

Water-borne Microorganisms (WMs) (related papers in the bottom row of TABLE I) refer to the safeguard of fishery production. In [8] an approach for wastewater bacteria recognition based on microscopic image analysis is described. The authors use 11 geometrical characteristics and an improved neural network for classification. Das et al. use a statistical signal modelling technique to distinguish seven kinds of WM shapes. They build two minimum distance pattern classifiers using geometrical features [9]. Ginoris et al. compare three WM classification methods on 22 classes of protozoan and metazoan [10]. In their experiment, geometrical features are used in the feature extraction process. Then, discriminant analysis, neural networks, and decision trees are employed for classification. Furthermore, in their cooperative work with Amaral et al., they have introduced another semi-automatic WM recognition method of protozoan exploiting both, a 2–D and a 3–D model to represent each protozoan [11], [12].

As described above, except our own previous work in [4], [5], no existing approaches address the EM classification problem. In addition, there are many other types of microorganisms, such as Food Microorganisms (FMs), Industrial Microorganisms (IMs), and Agricultural Microbials (AMs) that have not been undertaken computer-based analysis yet, although their manual interpretation plays an important role in many applications. FMs are used in food processing and storing, IMs are applied in industrial production, and AMs are considered to optimise the agricultural production. Therefore, it can be expected that conceptualisation and development of automated classification algorithms for other types of microorganisms will obtain increased scientific attention in the near future.

B. Selection of Image Analysis Methods for EM Classification

Image Segmentation: There exist different segmentation methods based either on pixel intensity levels or on image context. One popular intensity-based method is Otsu thresholding [13]. Another category of methods analyse first- and second-order derivatives and the local gradients (e.g., Sobel [14], Prewitt [15], Roberts [16], Laplacian of a Gaussian (LoG) [17], zero-crossing [18], and Canny edge detectors [19]). Further, the Watershed algorithm [20] simulates the topological features of geodesy and divides the image areas considering pixel values as altitudes. As pre- or post-processing techniques, morphological operations such as erosion and dilatation are often applied [21], [22].

Among all the methods mentioned above, we have selected the Sobel edge detector, because it is less sensitive to noise and easy to control. Our EM segmentation technique has been introduced in [5] and will not be described in this paper.

Feature Extraction: Because most of the EMs are colourless and transparent, it is nearly impossible to extract their colour and texture features. Shapes of EMs can be captured based on the optic boundary between light and shade. Hence, shape features are suitable for our research. There are some robust shape feature extraction methods, just as the Shape Context proposed in [23]. In our previous work [4], [5] we choose the following four shape features as a incipient attempt. The first one is the *Edge Histogram Descriptor*, which models the distribution of edge lengths. The second shape feature is the *Geometrical Feature*, including the EM perimeter, area, etc. The third one is the *Fourier Descriptor*, which is a contour-based shape feature [24] and represents a contour by applying the Fourier transform to distances between the centre and the points on the contour. The last shape feature is the *Internal Structure Histogram* (ISH) which characterises the structures of different EMs. This is an extension of Internal Structure Angles (ISAs) [25], each of which represents an angle defined by a combination of three points on the contour. We have extended ISAs by considering their distribution and modelling a histogram representation.

The *Bag-of-Visual-Words* (BoVW), which represents an image as a histogram modelling the distribution of features in local regions (i.e., local features) [26], is currently the most popular representation for image classification. In particular, the BoVW does not need image segmentation because it considers the statistics of many local features. In other words, an image showing a certain EM can be correctly classified if some of local features represent characteristic regions of the EM. However, our preliminary experiment using SIFT (Scale-Invariant Feature Transformation) features [27] showed that the BoVW does not work well for EM classification. Here, the BoVW of each image was created by assigning SIFT features at every sixth pixels to one of 1000 characteristic SIFT features (i.e., visual words). The mean of classification performances (average precisions) for 10 classes turned out to be only 51.3% which is significantly lower than for the new shape descriptor introduced in this paper. One main reason is that the BoVW is just a collection of local features describing small regions of an EM, and cannot describe its overall region. Thus, in this paper, we use the shape feature as a description of the overall region of an EM.

While in our previous work [4], [5] known feature extraction techniques with little extensions have been applied, in this paper a completely new and sophisticated shape descriptor is proposed (Section III).

Classification: Because our features are high-dimensional, we have selected the Support Vector Machine (SVM) as a classifier. Compared to the SVM, similarity-based classifiers like k-Nearest Neighbour and probability-based classification methods like Naive Bayes do not work well for high-dimensional features. Similarity-based classifiers fail to appropriately measure the similarity values for high-dimensional feature spaces due to many irrelevant dimensions unnecessarily taken into consideration. Probability-based classifiers need a large number of image examples to appropriately estimate probabilistic distributions in high-dimensional feature spaces [28]. However, due to the working habit of environmental researchers, who only keep a small number of typical microscopic images, it is difficult to collect a large and statistically relevant set of EM images for training.

For these reasons, we have selected an SVM which extracts a decision boundary between images of different EM classes

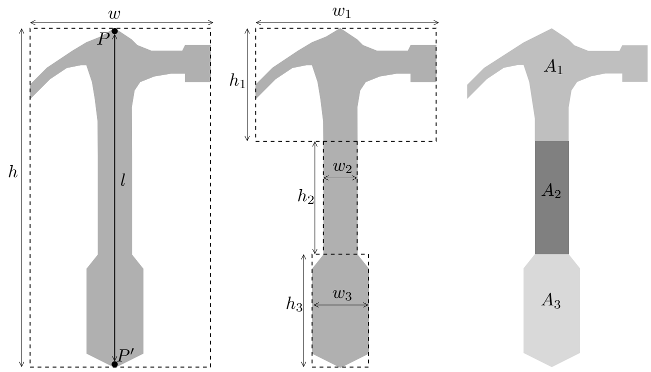

Fig. 1: Shape bounding box and equally high sub-boxes ($h_1 = h_2 = h_3$) used for feature extraction; A_1, A_2, and A_3 are the areas of the top, middle, and bottom sub-objects, respectively.

based on the margin maximisation principle. Due to this principle, the generalisation error of the SVM is theoretically independent of the number of feature dimensions [29]. Furthermore, a complex (non-linear) decision boundary can be extracted using a non-linear SVM.

III. Object Representation

Prior to feature extraction, we normalise the orientation of each object by rotating it so that the straight line connecting its two maximally distant contour points becomes vertical and the majority of contour points lies on the right side of this line (see Fig. 1)

An object shape is described by a 9-dimensional feature vector $\boldsymbol{c}'$. For this, we use the bounding box of the whole shape as well as the three equally high sub-boxes shown in Fig. 1. The first element c'_1 of the feature vector expresses the length of the object contour. The remaining elements are computed as follows:

$$\begin{aligned} c'_2 &= \tfrac{h}{w} & c'_3 &= \tfrac{h_1}{w_1} \\ c'_4 &= \tfrac{h_2}{w_2} & c'_5 &= \tfrac{h_3}{w_3} \\ c'_6 &= \tfrac{A_3}{A_1} & c'_7 &= \tfrac{A_2}{A_1} \\ c'_8 &= A_1 + A_2 + A_3 & c'_9 &= l \end{aligned} \qquad (1)$$

The selection of these features is not only based on the integration of geometric and topological features robust to shape deformation. But also we give attention to the speed of feature generation. Subsequently, we perform two feature normalisation steps. First, in order to ensure scale invariance, we divide the elements of the feature vector by a half of the bounding box perimeter:

$$\boldsymbol{c}^\star = \frac{\boldsymbol{c}'}{w+h} = (c^\star_1, c^\star_2, \ldots, c^\star_9)^{\mathrm{T}} \quad . \qquad (2)$$

Second, we linearly scale the feature values to the range $[0, 1]$:

$$\boldsymbol{c} = \frac{\boldsymbol{c}^\star - \min\{c^\star_1, \ldots, c^\star_9\}}{\max\{c^\star_1, \ldots, c^\star_9\} - \min\{c^\star_1, \ldots, c^\star_9\}} \quad . \qquad (3)$$

The scaling is needed for the Support Vector Machines applied in the classification step (Section IV). The main advantage of scaling is to avoid attributes in greater numeric ranges dominating those in smaller numeric ranges. Another advantage is to avoid numerical difficulties during the calculation. Because kernel values usually depend on the inner products of feature vectors (e.g., the linear kernel and the polynomial kernel), large attribute values might cause numerical problems.

IV. Classification

We have decided to use a Support Vector Machine (SVM) for classification to avoid the problems described in Section II. SVM is extracting a decision boundary between different classes of objects using the principle of maximising the margin. This leads to an generalisation error which is independent of the number of feature dimensions [29]. Using a non-linear SVM we can extract a complex (non-linear) decision boundary. In this process, microscopic images in a high-dimensional feature space are mapped into a higher-dimensional feature space using a kernel trick.

In our work, we applied a multi-class Support Vector Machine (mSVM) using its one-against-one (1vs1) version which works with a voting strategy. It uses a two-class SVM for each pair from a set of all considered classes $\{\omega_1, \omega_2, \ldots, \omega_K\}$. Thus, if there are K classes in total, $K(K-1)/2$ two-class classifiers have to be used. First, a sample pattern (query pattern) is classified using all these two-class SVMs. The final classification result is determined by counting to which class the sample pattern has been assigned most frequently.

V. Experimental Results

In this section, we first describe the experimental setting (Section V-A) and then comparatively evaluate our new shape feature descriptor in an EM classification scenario (Section V-B).

A. Experimental Setting

For experiments, we used a real dataset acquired in environmental laboratories of the University of Science and Technology Beijing. It contains ten classes of environmental microorganisms ω_1, ω_2, …, ω_{10}. Each class is represented by twenty microscopic images. We segmented the images manually and semi-automatically with the method introduced in [5]. Examples of EM original images as well as manually segmented and semi-automatically segmented EM images can be seen in Fig. 2. For supervised training, we randomly selected 10 manually segmented samples from each class. For testing either the 10 remaining manually segmented images or all 20 semi-automatically segmented samples from each class have been used. In order to increase statistical relevance, we repeated the selection process 10 times which led to 10 different training datasets. The test datasets changed only for experiments with manually segmented images. Experiments were performed for all these datasets and mean recognition rates were considered for evaluation.

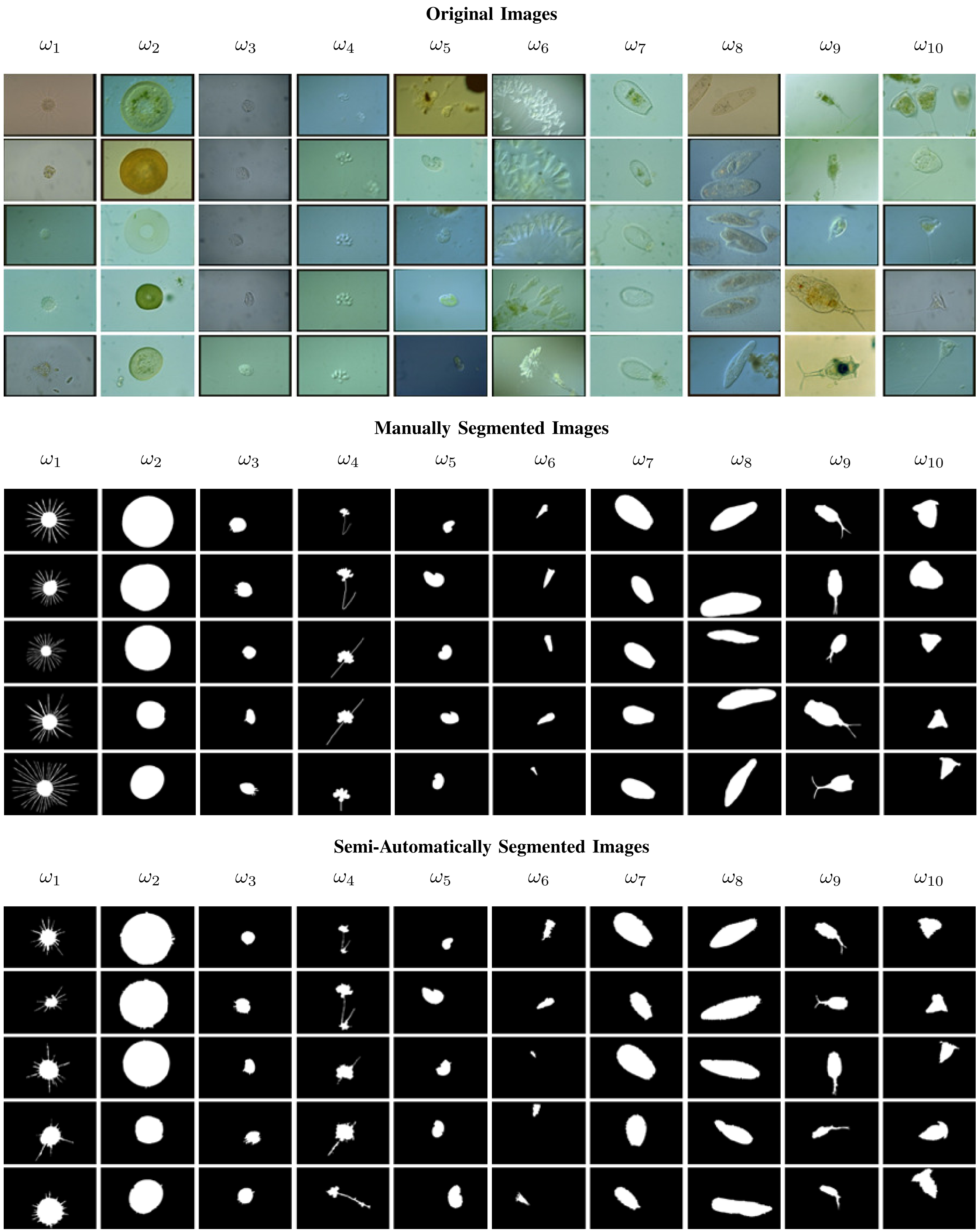

Fig. 2: Examples of original, manually segmented, and semi-automatically segmented EM images of all 10 classes.

B. Evaluation of the New Feature Descriptor

As one can see in TABLE II, the usage of the new shape descriptor introduced in this work led to better results in comparison to our previous work [4], [5] for both, the manually segmented and the semi-automatically segmented EM images. While for manually segmented images the overall classification rate increased from 89.7% to 92.5%, the benefits of the new feature extraction technique are even more significant for semi-automatically segmented images, namely from 66% to 79.5%, respectively. This result is very promising in terms of a possible real application of the system. In the real application the microorganisms are not expected to be fully-automatically recognised by a computer-based system. Environmental laboratory engineers would rather prefer to work with a recommendation system working in a content-based image retrieval manner. Having obtained a classification rate of 79.5% let us believe in this solution and encourages us to plan future work in this research area (see Section VI). However, as we can see from TABLE II, there are errors in approximately 20% of the classification rate for Semi-automatically segmented images with our proposed feature space. This is due to the misclassification for class ω_4 and ω_6. As shown in in Fig. 2, the shapes of ω_4 and ω_6 are quite unstable. Moreover, comparing results of ω_6 between two segmentation methods with our proposed features, there are only 5 misclassification in Manually Segmented Images, but 54 misclassification in Semi-Automatically Images. This tells us that the segmentation method is another reason for misclassification.

VI. Conclusion and Future Work

In this paper, we described an EM classification approach based on content-based image analysis techniques. Our system consists of three main phases: semi-automatic segmentation, shape description, and classification. The semi-automatic segmentation based on the Sobel edge detector proposed in our previous work [5] is used to extract EM regions in noisy and low-contrast microscopic images. The most important scientific contribution of this paper, in comparison to the state-of-the-art and to our previous publications in this field [4], [5] lies in the introduction of a completely new and very robust 2D feature descriptor for EM shapes. Experimental results certify the effectiveness and practicability of our automatic EM classification system emphasising the benefits achieved with the new shape descriptor proposed in this work. The classification rate for manually segmented EM images increased from 89.7% (for features used in [4]) to 92.5% for our new feature introduced in this paper. The improvement for semi-automatically segmented images is even more convincing - from 66% to 79.5%, respectively.

Due to the very promising results, our system can be considered to possess great potential towards the real-world application of EM recognition. However, to make it more effective, we will address the following three issues in the future. First, there exist many classes of EMs, much more than we have considered in this article. Therefore, we plan to create a much larger dataset of microscopic EM images and use it to test the generality of our system. Second, we aim at developing a full-automatic EM classification method. Our current experimental results show that a full-automatic method leads to inaccurate segmentation results. Thus, we plan to use multiple-instance learning to determine useful regions for EM classification among inaccurate and over-segmented EM areas [30]. Finally, real-time processing is important for a real-world application, where an EM image has to be examined and compared to various EM classes. Therefore, we will develop a method which utilises the hierarchical relationship among EM classes and avoids comparing an EM image to classes that are obviously irrelevant.

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Classification Rates for Manually Segmented Images [%]

Results for Features Proposed in Our Previous Work [4]

	ω_1	ω_2	ω_3	ω_4	ω_5	ω_6	ω_7	ω_8	ω_9	ω_{10}	
ω_1	**99**	0	0	1	0	0	0	0	0	0	99
ω_2	6	**90**	0	2	0	0	0	0	0	2	90
ω_3	0	0	**84**	6	2	2	0	0	0	6	84
ω_4	2	0	5	**93**	0	0	0	0	0	0	93
ω_5	0	0	4	0	**93**	3	0	0	0	0	93
ω_6	0	0	0	4	4	**92**	0	0	0	0	92
ω_7	1	0	0	2	0	0	**89**	0	0	8	89
ω_8	7	0	0	1	0	0	0	**89**	3	0	89
ω_9	2	0	0	4	0	0	0	2	**90**	2	90
ω_{10}	3	1	7	0	4	0	7	0	0	**78**	78
μ											**89.7%**

Results for Features Introduced in this Paper

	ω_1	ω_2	ω_3	ω_4	ω_5	ω_6	ω_7	ω_8	ω_9	ω_{10}	%
ω_1	**93**	0	0	0	0	0	7	0	0	0	93
ω_2	0	**100**	0	0	0	0	0	0	0	0	100
ω_3	1	0	**77**	0	18	0	0	0	0	4	77
ω_4	0	0	5	**84**	0	0	0	2	9	0	84
ω_5	0	0	7	0	**93**	0	0	0	0	0	93
ω_6	0	0	0	0	5	**95**	0	0	0	0	95
ω_7	0	0	0	0	1	0	**99**	0	0	0	99
ω_8	0	0	0	0	0	0	0	**100**	0	0	100
ω_9	0	0	0	0	0	0	0	0	**100**	0	100
ω_{10}	0	0	0	0	1	0	15	0	0	**84**	84
μ											**92.5%**

Classification Rates for Semi-Automatically Segmented Images [%]

Results for Features Proposed in Our Previous Work [4], [5]

	ω_1	ω_2	ω_3	ω_4	ω_5	ω_6	ω_7	ω_8	ω_9	ω_{10}	
ω_1	**70**	0	0	30	0	0	0	0	0	0	70
ω_2	10	**90**	0	0	0	0	0	0	0	0	90
ω_3	0	0	**100**	0	0	0	0	0	0	0	100
ω_4	0	0	15	**80**	0	5	0	0	0	0	80
ω_5	0	0	20	0	**55**	0	0	0	0	25	55
ω_6	0	0	0	55	0	**40**	0	0	5	0	40
ω_7	5	0	0	0	0	0	**5**	15	25	50	5
ω_8	15	0	0	0	0	0	0	**75**	10	0	75
ω_9	0	0	0	5	0	0	0	0	**95**	0	95
ω_{10}	20	0	0	0	15	0	0	0	15	**50**	50
μ											**66.0%**

Results for Features Introduced in this Paper

	ω_1	ω_2	ω_3	ω_4	ω_5	ω_6	ω_7	ω_8	ω_9	ω_{10}	
ω_1	**50**	5	25	5	0	0	0	0	5	10	50
ω_2	0	**100**	0	0	0	0	0	0	0	0	100
ω_3	0	0	**85**	0	15	0	0	0	0	0	85
ω_4	0	0	25	**40**	5	15	0	5	5	5	40
ω_5	0	0	15	0	**85**	0	0	0	0	0	85
ω_6	0	0	5	0	40	**55**	0	0	0	0	55
ω_7	0	0	0	0	5	0	**95**	0	0	0	95
ω_8	0	0	0	0	0	0	0	**100**	0	0	100
ω_9	0	0	0	0	0	0	0	0	**100**	0	100
ω_{10}	0	0	5	0	0	0	10	0	0	**85**	85
μ											**79.5%**

TABLE II: Classification rates for manually (first row) and semi-automatically (second row) segmented images achieved using the feature extraction technique introduced in our previous work [4], [5] (left) and the feature descriptor proposed in this paper (right). The new shape feature extraction technique possess significantly better discriminative properties.

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