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Analysis of Virus Textures in Transmission Electron Microscopy Images

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Abstract. In this paper we propose an ensemble of texture descriptors for analyzing virus textures in transmission electron microscopy images. Specifically, we present several novel multi-quinary (*MQ*) codings of local binary pattern (*LBP*) variants: the *MQ* version of the dense *LBP*, the *MQ* version of the rotation invariant co-occurrence among adjacent *LBPs*, and the *MQ* version of the *LBP* histogram Fourier. To reduce computation time as well as to improve performance, a feature selection approach is utilized to select the thresholds used in the *MQ* approaches. In addition, we propose new variants of descriptors where two histograms, instead of the standard one histogram, are produced for each descriptor. The two histograms (one for edge pixels and the other for non-edge pixels) are calculated for training two different *SVMs*, whose results are then combined by sum rule. We show that a bag of features approach works well with this problem. Our experiments, using a publicly available dataset of 1500 images with 15 classes and same protocol as in previous works, demonstrate the superiority of our new proposed ensemble of texture descriptors. The MATLAB code of our approach is available at https://www.dei.unipd.it/node/2357.

Keywords. texture descriptors, feature selection, multi-quinary coding, local binary patterns, ensemble of descriptors

1 Introduction

Transmission Electron Microscopy (TEM) (in particular, negative staining TEM) is considered the preferred technique for virus detection and identification, especially for unknown agents [4]. TEM is chosen despite high maintenance costs and the level of expertise required for manual inspection of images. A fundamental approach for characterizing viruses is to visualize their physical structure. Since TEM can be performed without any a priori knowledge of the pathogens present in a sample, it is not dependent on any organism-specific reagent [7]. This allows a broader examination of the sample than is available with other diagnostic tools such as detection of antigens or molecular diagnostic techniques.

In general viruses are classified on the basis of their morphology, chemical composition, and mode of replication. TEM analysis offers important morphological

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information about size and shape (such as whether a virus is icosahedral, helical, complex, or even morphologically non-descriptive [6]). Size and shape, however, are not qualities that in themselves are sufficient for confirming the identification of a virus. TEM also provides important detailed texture information, which can be exploited using modern texture analysis techniques [13], thereby providing the opportunity of automating TEM virus image classification. Automation of virus classification using TEM images is highly desirable because it would greatly reduce costs as well as the intra-/inter-operator variability of human expert assessments.

In the last two decades, there has been a resurgence of interest in machine vision on the topic of image texture. In the 1970s texture classification methods were focused primarily on the statistical analysis of images. Some important methods exploiting this approach include those based on the co-occurrence matrix [10] and filtering [22]. At the turn of the century, Ojala et al. [17] proposed a Local Binary Pattern (LBP) histogram for rotation invariant texture classification. LBP (and its variants) is a simple and efficient operator for describing the local image pattern and has achieved impressive classification results on many machine vision benchmark datasets, including those for virus image classification [13] and protein subcellular localization [19]. Moreover, several systems using LBP have been applied in real-world applications (see, for instance, [11] and [27]). In the last decade, LBP has distinguished itself from other texture descriptors by its simplicity, effectiveness, and robustness in detecting textural and structural information, becoming today one of the most widely researched methods for textual analysis.

Despite recent advances in image texture analysis, only a handful of papers have examined machine analysis of virus textures in TEM images. Discrimination of four icosahedral viruses are reported in [14], using ring filters in the Fourier power spectrum, and in [18], using higher order spectral features. In [23] a radial density profile (RDP) is applied to TEM images of cell sections to discriminate intensity variations between three maturation stages of human cytomegalovirus capsids. In [13] an ensemble is proposed that combines LBP and RDP to discriminate fifteen virus types, and in [15] an ensemble is proposed that combines several texture descriptors.

As in [15], we present an ensemble that combines several texture descriptors to classify TEM virus images. Several advancements, however, are introduced in this work:

- For three methods, we test for the first time their multi-quinary (MQ) coding versions [19]. The sequential forward floating selection (SFFS) [20] method is used to select a set of optimal parameters for building the MQ versions. The selection is useful for reducing computation time and for improving performance;
- We couple our texture descriptors with the idea proposed in [1] and applied to LBP and LTP. Instead of calculating a single histogram for each descriptor, we calculate two histograms: one for edge pixels and the other for non-edge pixels. Two different SVMs are then trained, one for each of the two histograms. Results are combined by sum rule;
- We show that a bag of feature approach, based on a perturbation technique for improving the performance, works well on this problem;
- We present a powerful ensemble for solving the virus image classification problem that is based on the novel approaches proposed in this paper and on methods reported in our previous work.

This paper is organized as follows. In section 2 we illustrate the descriptors used in our ensembles. In section 3 we present the results using our system on a publicly available dataset of 1500 images grouped into 15 different virus types (http://www.cb.uu.se/~gustaf/virustexture/index.html). We aim to show how our ensemble performs compared to previous approaches where the same dataset is used for assessing performance. In section 4 we conclude by also offering a few comments on results obtained in previous reports using the same dataset. Finally, we suggest possible future research lines of our approach.

2. Proposed Approaches

In this section we describe the following: the multi-quinary coding method we apply to a set of descriptors, the edge-based dual histogram idea, and the proposed bag of feature approach used in our experiments.

2.1. Multi quinary coding (MQ)

In this work we expand our previous efforts of applying multi-quinary coding (*MQ*) to descriptors by applying it to several local binary pattern (LBP) variants. In particular, we report the performance obtained by the following:

- MQ version of the dense LBP (DLBP) [26]. In section 3, *MDLB* is the label used for the multi-quinary coding of DLB;
- MQ version of the rotation invariant co-occurrence among adjacent LBPs (RI) [16]. In section 3, *MRI* is the label used for the multi-quinary coding of RI;
- MQ version of the LBP histogram Fourier (LHF) [2], In section 3, *MLHF* is the label used for the multi-quinary coding of LHF

To reduce computation time and to improve performance, the threshold couples used for the quinary codings are selected using *SFFS* [20], a feature selection approach where each potential feature is given a threshold couple that is then evaluated. In this way, we select the classifiers (each classifier is trained using a given threshold couple) to be combined by running *SFFS* with an objective function that minimizes the error rate of the sum rule of the classifiers trained using the selected threshold couple (evaluated using a 5-fold cross validation method on the training data).

The idea of quinary coding for LBP is proposed in [19], where it is applied to the canonical LBP operator [17], which assigns a binary label to each pixel of an image based on the local information extracted from a circular neighborhood of P pixels and radius R according to the following equation:

$$LBP(P,R) = \sum_{p=0}^{P-1} s\left(q_p - q_c\right) \cdot 2^p \tag{1}$$

where

$$s(x) = \begin{cases} 1, x \geq 0 \\ 0, x < 0 \end{cases}$$

Quinary coding is applied to LBP by introducing into the binary coding $s(x)$ two more thresholds (τ_1, τ_2), as follows:

$$s(x,\tau_1,\tau_2) = \begin{cases} 2, x \geq \tau_2 \\ 1, \tau_1 \leq x < \tau_2 \\ 0, -\tau_1 \leq x < \tau_1 \\ -1, -\tau_2 \leq x < -\tau_1 \\ -2, x < -\tau_2 \end{cases} \tag{2}$$

To reduce the verbosity of the quinary encoded labels assigned to each pixel of the image, the quinary labels are split into four sets of binary patterns, according to the binary function $b_c(x)$, $c \in \{-2,-1, 1,2\}$:

$$b_c(x) = \begin{cases} 1, & x = c \\ 0, & otherwise \end{cases} \tag{3}$$

These binary sets b_c are then used for extracting histograms (as in standard tested descriptors for LBP and LHF, etc.). The four histograms are then concatenated for training a SVM.

In our experiments, we have used more couples of thresholds than the standards reported in [19]. We chose sets of threshold couples according to $\tau_1=\{1,3,5,7,9,11,13,15\}$ and $\tau_2=\{\tau_1+2, ..., 19\}$. A subset of threshold couples is then selected by SFFS, as described above using the training data.

2.2. Edge (ED)

LBP Edge [1] represents an improvement of the LBP descriptor. It is based on the evidence that when observers stare at natural images, the most likely fixated locations are the high spatial frequency edges [3].

The original LBP Edge descriptor is obtained as follows:

1. Apply the canonical LBP to an image *I* to get the LBP Image (*LBPI*);
2. Detect edges in *I* using the Sobel edge detector. This results in 2 maps: the edge map (*E*) and the non-edge map (*NE*);
3. Compute two histograms, *H_E* and *H_NE*, by combining LBPI with *E* and LBPI with *NE*, respectively (see [1] for details);
4. Concatenate *H_E* and *H_NE* into one final histogram (the feature vector), where $H = [w_E \times H_E, w_{NE} \times H_{NE}]$, using the empirically determined weights w_E and w_{NE}, where $w_E > w_{NE}$ expresses the greater visual attention of the viewer on edge regions than in non-edge regions.

In [1], this idea is coupled with LBP and its ternary version, LTP. Here we couple their idea with more descriptors (see section 3 for details). Moreover, the two histograms *H_E* and *H_NE* are not combined into one feature vector but rather are used to train two different SVMs that are then combined by sum rule.

2.3. Bag of features approach (BOF)

In this paper we proposed a bag of feature approach based on perturbation techniques for building an ensemble of codebooks for improving performance. Due to lack of space, we do not describe the basic idea of bag of features, but refer the reader to [12] or [28] for more details.

In our experiments, we use a grid space approach, where each image is divided into overlapping subwindows. The size *st* of the subwindows is specified as a percentage *ps* of the original image taken at fixed steps, such that $st = min(ps \times l, ps \times h)/2$, where $l \times h$ is the size of the original image. In our final version, ps=12.5%.

We perform different projections, retaining different training subsets for building different projection matrices. For each projection matrix, a different clustering is performed and a different vocabulary is built and used for training a SVM.

A set of 50000 subwindows are randomly extracted from the training set (considering the different classes) and used to construct the principal component analysis (PCA) matrix. This step is iterated several times (five times retaining 99% of the variance, five times retaining 98% of the variance, and five times retaining 99.9% of the variance); for each iteration, a different vocabulary is created and a different SVM is trained. The results of the 15 SVMs are then combined by sum rule.

A different set of codebooks is created for each class of the dataset: for each 50 images of each class, a codebook is built by k-means clustering, with *k* randomly selected between 10 and 40 in order to increase the diversity among the codebooks built in the different iterations. The final vocabulary is obtained by concatenating the codebooks over all classes. Each subwindow is assigned to one element of the vocabulary according to the minimum Euclidean distance criterion (for further details, see the MATLAB code available at https://www.dei.unipd.it/node/2357).

3. Experimental Results

The "object scale" virus dataset tested in [13] (available at http://www.cb.uu.se/~gustaf/virustexture/index.html) is used for assessing the performance of our system. This dataset contains 1500 images of 15 virus types. In our experiments, we used the same 10-fold cross validation testing protocol applied in [13] and in our previous work [15] (the indices used in the 10-fold cross-validation procedure are available at http://www.cb.uu.se/~gustaf/virustexture/index.html).

For comparison purposes, table 1, presents the results of the most interesting methods reported in our previous work[2,3] [15]:

- NewH, a method based on the co-occurrence matrix, for details see [15];
- LBP, standard local binary pattern;
- LTP, standard local ternary pattern;
- MLQP, standard multithreshold local quinary pattern [19];
- PLB, pyramid LBP [21];

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[2] https://www.researchgate.net/publication/260210011_Virus_image_classification_using_different_text ure_descriptors.

[3] For all the tested approaches the uniform rotation invariant LBP mapping is used and considering the (P=8,R=1) and (P=16,R=2) neighborhoods.

- PLQ, the variant of PLB where the quinary coding is used instead of the standard binary coding;
- NTB, noise tolerant LBP [5];
- NTQ, the variant of NTB where the quinary coding is used instead of the standard binary coding;
- DLB, discriminative LBP [8];
- DLQ, the variant of DLB where the quinary coding is used instead of the standard binary coding;
- LCP, local configuration pattern [9];
- MLC, the variant of LCP where the quinary coding is used instead of the standard binary coding;
- Morph, the method proposed in [24] (with the exclusion of the Haralick features [15]);
- FRDP [13], the best performing method in the “object scale dataset” (i.e. the same used in this work) reported in [13]. We use the original code shared by the authors coupled with SVM.
- FUSION, fusion by sum rule among NewH, MLC, Morph and FRDP (the best 4 methods, each belonging to a different type of descriptors).

Table 1. Performances obtained by the methods reported in [15] (note: we report the mean accuracy among the classes and not the median as in [13].

LBP	LTP	MLQP	PLB	PLQ	NTB	NTQ	Fusion
57.6	58.5	70.0	64.0	70.1	49.9	68.5	**80.7**
DLB	**DLQ**	**LCP**	**MLC**	**NewH**	**Morph**	**FRDP**	
55.3	71.8	62.7	73.3	71.7	71.7	70.0	

In table 2, we report the most interesting results obtained coupling the idea of LBP Edge with several descriptors. It is interesting to note that LBP Edge improves the performance of all the tested descriptors. In each cell of table 2 two values are reported: first the standard descriptor accuracy and second the accuracy obtained coupling the descriptor with the LBP Edge idea. In table 2 we label the descriptor coupled with LBP Edge as *X_ED*, where *X* is the short name of the descriptor (e.g. LBP_ED).

Notice that in this work, the uniform mapping is used and the descriptors are extracted considering the (P=8, R=1) and (P=16, R=2) neighborhoods for LBP, LTP, LHF, and DLBP. Moreover, it is important to note that we did not use the masks available at http://www.cb.uu.se/~gustaf/virustexture/index.html for extracting the foreground; rather the entire image is used for extracting the features. For this reason, LBP and LTP obtain a performance that differs from that reported in our previous work.

Table 2. Accuracy obtained by combining the LBP Edge idea with several descriptors.

LHF	LBP	LTP	DLBP	RI	LPQ
59.7/64.5	58.1/61.4	63.2/65.1	70.1/71.6	79.6/79.7	63.3/69.3

In table 3 we report the performance obtained by the BOF approach combined with different descriptors. Due to computation time issues, we tested BOF coupled with only four different descriptors: LHF, LBP, LPQ and DLBP. In each cell of table 3 two values are reported. The first is the accuracy obtained when only a single vocabulary is used (i.e., only the first iteration of the system detailed in section 2.3). The second is the accuracy obtained using the ensemble of 15 SVMs as detailed in section 2.3. The method named BF in table 3 is the fusion by sum rule of the four BOF approaches. It is clear that BF combined with different descriptors outperforms BOF based on a single descriptor.

Table 3. Performance obtained using the BOF approach.

BOF LHF	BOF LBP	BOF LTP	BOF DLBP	BF
47.5/48.8	53.3/57.8	51.3/55.3	61.3/61.4	63.0/**69.0**

In table 4 we report the performance of the MQ variant proposed in this paper. Moreover, we report the performance of the following:

- ME: the shape- and appearance-based descriptor proposed in [25];
- NewF: the fusion[4] of the best approaches by sum rule of *BF* + *MDLB* + *MRI* + *ME* + *NewH* + *MLC* + *Morph* + *FRDP* + *DLBP_ED* + *RI_ED* + *LPQ_ED*.

Table 4. Comparison among the MQ based methods and the novel ensemble.

LHF	MLHF	DLBP	MDLB	RI	MRI	ME	NewF
59.7	63.5	70.1	70.8	79.6/	77.6	66.7	**85.7**

The results reported in this paper differ from those reported in our previous work: MQ does not prove to be very effective as it does not improve the performance of RI and is only marginally useful in LHF and DLBP. However, the proposed new ensemble NewF works best: it is simply built by combining the most performing descriptors according to the sum rule. In building the ensemble, it should be noted that to avoid overfitting in the dataset we did not use an optimization algorithm. NewF obtains a mean accuracy of 85.7% and a median accuracy of 86.0% outperforming all previous works based on the same “object scale" dataset. For comparison, our best ensemble used in [15] obtained a mean accuracy of 80.7% only.

4. Conclusion

In this work we expand our previous study on virus image classification by exploring novel variants of state-of-the-art descriptors. We show that fusion by simple sum rule among the best approaches obtains the best reported performance on the “object scale” dataset, beating the performance obtained in [13] and [15] (we used the same 10-fold cross validation protocol on the “object scale” dataset used in the original paper).

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[4] Before the fusion, the scores of each method are normalized to mean 0 and standard deviation 1

Whereas a mean accuracy of 73.8%, along with a median accuracy of 79.0%, is reported in [13] (as provided in a personal communication with the authors), the ensemble we propose in this paper obtains a mean accuracy of 85.7% and a median accuracy of 86.0%.

The best result reported in [13] is a median accuracy of 87% when descriptors obtained from both the “fixed scale" (which is not publicly available) and “object scale" datasets are combined (see [13] for details). In this work we obtain a similar performance using the “object scale" dataset only.

In conclusion, the proposed ensemble provides high performances in the “object scale” dataset by exploiting: (i) state of art texture descriptors, (ii) the edge idea, i.e. taking particular advantage of the edge pixels in the images by extracting two histograms and (iii) the BOF approach. In the future, we plan on testing the proposed system and the proposed variants based on *MQ* coding and *LBP edge* on other applications with large datasets.

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