General Purpose (GenP) bioimage ensemble of Handcrafted and Learned Features with Data Augmentation

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Abstract

Bioimage classification plays a crucial role in many biological problems. Here we present a new General Purpose (GenP) ensemble that boosts performance by combining local features, dense sampling features, and deep learning approaches. We propose an ensemble of deep learning methods built using different criteria (different batch sizes, learning rates, topologies, and data augmentation methods). One of the contributions of this paper is the proposal of new methods of data augmentation based on feature transforms (principal component analysis/discrete cosine transform) that boost performance of Convolutional Neural Networks (CNNs). Each handcrafted descriptor is used to train a different Support Vector Machine (SVM), and the different SVMs are combined with the ensemble of CNNs. Our method is evaluated on a diverse set of bioimage classification problems. Results demonstrate that the proposed GenP bioimage ensemble obtains state-of-the-art performance without any ad-hoc dataset tuning of parameters (avoiding the risk of overfitting/overtraining).

Keywords: Microscopy imaging, Classification, Deep Learning, Support Vector Machine.

1 Introduction

Biomedical research is increasingly dependent on computer vision and machine learning in the discovery of new knowledge and methods of diagnosis. Storing, retrieving, and analyzing high dimensional biological images has become critical, in part because of the enormous amounts of data generated by recent advances in microscopy imaging technologies, such as automated brightfield microscopes and confocal microscopy [1, 2]. Automated image analysis has become an indispensable tool not only for handling the mass of data collected by these devices but also for
providing researchers consistent and objective analysis, as demonstrated in recent research in such areas as cell phenotype recognition, subcellular localization, and histopathological classification [3-6].

Because computer vision and image classification rely on powerful methods for extracting highly discriminative feature sets, a major area of research in these domains has focused on generating ever better methods for extracting powerful descriptors. Until recently, however, most bioimage research has concentrated on the problem of segmentation [7] with little attention devoted to investigating the discriminative power of texture descriptors—even though it has been shown that extracting highly discriminative texture descriptors can circumvent the problem of segmentation [7-9]. Some whole image methods of note that have been proposed in the literature include [10-12], and some popular descriptors used in automatic bioimage classification include traditional Gabor filters [13] and Haralick’s famous texture features [14].

More recent descriptors applied to automatic bioimage analysis include such powerful handcrafted descriptors as the scale-invariant feature transform (SIFT) and local binary patterns (LBP) [15-17]. Handcrafted descriptors are those that are designed by researchers to extract specific image characteristics. The extraction of handcrafted features is typically accomplished as follows: characteristic regions of an image are located by a keypoint detector, and these regions are described by a vector of measurements (which is the descriptor) that depends on the specific image characteristics under consideration. The extracted set of descriptors is then used to train a classifier, such as the Support Vector Machine (SVM) [18].

In contrast to handcrafted descriptors are nonhancrafted or learned descriptors, which, as indicated by their name, are automatically learned by a classifier system. Learned descriptors have only recently been explored in bioimage classification. Vu et al. [19], for instance, proposed an
automatic feature discovery method that uses class-specific dictionaries for the diagnosis of ovarian carcinomas, and Otalora et al. [20] combined both handcrafted and learned descriptors to discriminate irregularities in brain cells (the authors proposed a system that combines an unsupervised feature learning method with learned linear combinations of Riesz wavelets calculated at several orders and scales to capture the granularity of multiscale rotation-covariant information).

Within the last few years, some innovative nonhandcrafted approaches have been proposed that exploit deep learners [21], such as the Convolutional Neural Network (CNN) that has revolutionized image classification. It appears that deep learners analyze input images via the different layers in the architecture by evaluating sets of features learned directly from observations of the training images [22], some of which are even thought to preprocess images using a pyramidal approach [23]. When deep neural networks, such as CNN, are trained on a set of images for a specific classification problem, features extracted by the shallowest layers (those nearest to the classification layer) are strongly dependent on the training set, but the first layer features resemble Gabor filters or color blobs that tend to be transferable to many other classification problems [24]. This discovery has been exploited by bioimage researchers who have used CNN [25] or ensembles of CNNs [26] as feature extractors; the resulting learned features are then treated like SIFT and LBP and become the input to other types of classifiers, such as SVM.

A large image dataset is necessary for training deep learners. This poses a problem since the required size is much larger than what is available in most bioimage datasets, which are often difficult and expensive to acquire. Employing standard data augmentation techniques is one popular method for increasing both the size and diversity of images in small datasets, and these techniques have been used extensively in the analysis of medical and biological images [27].
augmentation also combats overfitting CNNs and is often used to improve performance. For example, Rakhlin et al. [28] were able to accurately detect breast cancer in a set of histology images containing less than 100 images per class by combining pretrained deep network architectures with multiple augmentation techniques.

The most common methods of image data augmentation involve reflection, translation, and rotation [29-32] as these augmentations generate different representations of the same sample. Different representations of a given image can also be constructed by altering contrast, saturation, and brightness [29, 31, 32]. Yet another common technique is PCA jittering, which accentuates the most relevant features of an image by adding to it some of its principal components multiplied by a small number [29, 31]. Most deep learning frameworks implement a limited set of basic image transforms. Recently, however, libraries of fast augmentation methods have been developed, such as Albumentaions [33], which provides a large number of image transforms along with an easy-to-use wrapper around other augmentation libraries.

Very specific problem-dependent augmentation methods can also be applied to expand small datasets. For example, Ding et al. [34] replicated speckle noise, a common artifact in SAR images, by applying random pointwise multiplications to images, and Castro et al. [35] reproduced different stretchings of the human body by creating elastic deformations of breast cancer images. Operations like elastic transforms and grid distortions are useful in medical imaging, where non-rigid structures that have shape variations are quite common [33].

Another very recent method for enlarging small datasets uses Generative Adversarial Networks (GANs) to synthesize new images that are different from those contained in the original dataset [36-38]. GANs are based on an adversarial game between two neural networks: a generator network $G$ that produces synthetic samples given some random noise, and a discriminator network
that distinguishes between the generator’s synthesized image and true image. Because GANs generate new images on a separately trained network, they produce, unlike data augmentation techniques, a unique yet relevant set of new images.

In this paper, we present a new General Purpose (GenP) bioimage classification method that combines both handcrafted and learned descriptors. Ideally, a GenP image classification system should be capable of handling a broad range of different image classification tasks. In other words, a GenP system should work well on any image problem in a given domain and require little (if any) parameter tuning. A GenP system should also perform competitively well against other systems that have been optimized for very specific image classification problems.

Our new bioimage GenP system combines handcrafted features and deep learning methods to obtain a high degree of generalizability across a wide range of bioimage datasets. A representative set of powerful handcrafted descriptors are individually trained on a separate SVM, and the set of SVMs is combined by sum rule. We also propose a very high-performing ensemble of CNNs, where the decisions of the separate CNNs are likewise combined by sum rule. Finally, the CNN ensemble is combined with the SVMs trained on the handcrafted descriptors.

The ensemble of CNNs is built as follows: different CNN topologies are investigated that use two different learning rates \(\{0.001, 0.0001\}\), four different batch sizes \(\{10, 30, 50, 70\}\), and set of different data augmentation approaches, two of which are proposed here for the first time. The new approaches are based on two well-known feature transforms: the discrete cosine transform (DCT) and principal component analysis (PCA). Both approaches are based on projecting the original image onto the DCT/PCA subspace, which perturbates the retro-projection from the subspace to the original space.

The main contribution and focus of this paper is on the proposal of new approaches for data
augmentation that are based on PCA and DCT. In addition, we show that our new data augmentation methods, combined with other approaches, can be used to build an ensemble of CNNs. Lastly, we demonstrate that an ensemble combining different augmentation methods with handcrafted and learned descriptors produces a powerful GenP bioimage classifier that not only works across a large set of benchmark datasets but also obtains state-of-the-art performance. Indeed, one of the advantages of our proposed ensemble, compared with others published in the literature, is that the GenP system proposed here requires no fine-tuning of parameters on new bioimage datasets. The image representation methods used in our system are general and designed to work efficiently on many bioimage classification problems. What this means is that the training phase using our GenP system on a new bioimage dataset would most likely be limited to the classification step.

To prove that our system is highly generalizable, we evaluate our method on a diverse set of bioimage classification problems, each represented by a benchmark dataset. Some of these datasets are publicly available in the IICBU 2008 database, and each bioimage task represents a typical subcellular, cellular, and tissue level classification problem. Results show that the proposed GenP bioimage ensemble obtains state-of-the-art performance without any ad hoc dataset tuning of parameters.

2 Handcrafted Methods

As mentioned in the introduction, our GenP bioimage ensemble combines both handcrafted and learned descriptors as well as some traditional and novel augmentation methods. Because the handcrafted descriptors that were tested when building our bioimage system have been extensively described in the literature and are familiar to most researchers in bioimage classification, we only very briefly describe them in Table 1, where we also provide the parameter settings used for each
descriptor. In datasets where color images are available, each descriptor is extracted from each color channel; and, for each of the three channels, a different SVM is trained, with the set of SVMs combined by sum rule.

**Table 1. Summary of Texture Descriptors**

| Acronym | Brief Description of Descriptor and Parameter Settings | Source |
|---------|------------------------------------------------------|--------|
| LTP     | Multiscale Uniform Local Ternary Patterns (an extension of LBP [39]) with two \((radius, neighboring points)\) configurations: \((1, 8)\) and \((2, 16)\). | [40] |
| MLPQ    | Multithreshold Local Phase Quantization are sets of LPQ descriptors that vary the filter sizes, the scalar frequency, and the correlation coefficient between adjacent pixel values. Each extracted descriptor is used to train a different SVM. | [41] |
| CLBP    | Completed LBP with two \((radius, neighboring points)\) configurations: \((1,8)\) and \((2,16)\). | [42] |
| RIC     | Multiscale Rotation Invariant Co-occurrence of Adjacent LBP with \(radius \in \{1, 2, 4\}\). | [43] |
| GOLD    | Gaussians of Local Descriptors. Here we train a different SVM from each region of the spatial pyramid and combine them by sum rule. We use one level spatial pyramid decomposition: the decomposition consists of the entire image, followed by level one, where the image is subdivided into four quadrants | [44] |
| COL     | A simple and compact color descriptor | [45] |
| AHP     | Adaptive Hybrid Pattern combines \(i\) a Hybrid Texture Model (HTD) composed of local primitive features and a global spatial structure and \(ii\) an adaptive quantization algorithm (AQA) to improve noise robustness. We fixed quantization level = 5; we used 2 \((radius, neighboring points)\) configurations: \((1, 8)\) and \((2, 16)\). | [46] |
| FBSIF   | Extension of the canonical Binarized Statistical Image Features (BIF) by varying the parameters of filter size \((\text{SIZE}_{\text{BSIF}}, size \in \{3, 5, 7, 9, 11\})\) and a threshold \((\text{th})\) for binarizing \((\text{FULL}_{\text{BSIF}}, th \in \{-9, -6, -3, 0, 3, 6, 9\})\). | [47] |
| LET     | A simple but effective representation that encodes the joint information within an image across feature and scale spaces. We use the default values available in the MATLAB toolbox. | [48] |
| MOR     | Morphological descriptor is a set of measures extracted from a segmented version of the image, including the aspect ratio, number of objects, area, perimeter, eccentricity, and other measures. | [49] |
| CLM     | CodebookLess Model. We use the ensemble called CLoVo_3 in [50] based on e-SFT, PCA for dimensionality reduction, and one-vs-all SVM for the training phase. | [51] |
| ETAS    | We utilized Threshold Adjacency Statistics from a novel perspective to enhance its discrimination power and efficiency. In this connection, we utilized seven threshold ranges to produce seven distinct feature spaces, which are then used to train a single SVM. | [52] |

## 2 Deep Learning Methods

CNNs are a class of deep feed-forward neural networks composed of interconnected neurons arranged in three dimensions (width, height and depth). Every layer in a CNN transforms a 3D input volume into a 3D output volume of neuronal activations. There are five classes of layers in a CNN: convolutional (CONV), activation (ACT), pooling (POOL), Fully-Connected (FC), and
In this work, we evaluate ensembles composed of the following CNN architectures pretrained on the ImageNet database:

- AlexNet [29]: the first GPU-implementation of a CNN and winner of the 2012 ImageNet Large Scale Visual Recognition Challenge;
- GoogleNet [53]: CNN that includes a module called an inception module that approximates a sparse CNN with a normal dense construction;
- VGGNet16 & VGGNet19 [54]: architectures from the VGG group that improves AlexNet by replacing large kernel-sized filters with multiple 3X3 kernel-sized filters;
- ResNet50 [55]: CNN network 50 layers deep available in MATLAB;
- DenseNet: [56]: logical extension of ResNet that connects each layer to every other layer;

Each CNN is finetuned on each of the tested dataset. Finetuning a CNN is a procedure that essentially continues the training process of a given pretrained network so that it learns a new classification problem. A CNN that produces random results on the training data (fails to converge) is excluded from the ensemble. It is not always possible to train a CNN with a large batch size, in which case a “GPU out of memory” error message results, and that CNN configuration is excluded as well.

3 DATA AUGMENTATION (DA) METHODS

It was noted in the introduction that not only is DA a highly effective way to enlarge a small training set, but it also enhances performance and reduces overfitting during CNN training. The basic idea behind DA is to apply various transformations and deformations to the labeled data to
produce new samples in the training set.

Our basic DA work flow can be described as follows. At the beginning of each epoch, we randomly transform each image in a given dataset with some basic preprocessing methods, such as rotation and reflection. Following this random preprocessing stage, four different data augmentation protocols (App1-4) are applied:

App1: The image is reflected in the left-right direction with 50% probability.

App2: The image is randomly reflected in both the left-right and the top-bottom directions. In addition, App2 linearly scales the image along both axes by two different factors that are randomly sampled from the uniform distribution in [1, 2].

App3: Combines all the transformations in App2 and adds image rotation and translation in both directions. The rotation is done using an angle that is randomly sampled from the interval [-10, 10], while the translation consists in shifting the image by a certain number of pixels randomly sampled from [0, 5].

App4: Extends App3 by also applying vertical and horizontal shear, with the shear angles randomly sampled from the interval [0, 30].

In addition to the four different data augmentation protocols, we applied two new approaches presented for the first time here that are based on two common feature transforms: Discrete Cosine Transform (DCT) and Principal Component Analysis (PCA). Both these transforms are based on the projection of the original image onto the DCT/PCA subspace and perturbing the retro projection from the subspace to the original space. Our two new DA approaches are labelled as follows:

App5: DA approach based on PCA.
App6: DA approach based on DCT.

PCA [57] is a popular method for image compression, so it is often used as an unsupervised dimensionality reduction method. Computationally cheaper to compute than PCA, DCT maps feature vectors into a smaller number of uncorrelated directions calculated to preserve the global Euclidean structure. Like DCT, PCA also extracts an orthogonal projection matrix but in such a way that the variance of the projected vectors is maximized. DCT provides a good compromise between information packing and computational complexity [58]. Computational complexity is reduced because DCT is not data dependent, unlike PCA, which needs the eigenvalue decomposition of the data covariance matrix. DCT components are also very small in magnitude since most of the salient information exists in the coefficients with low frequencies. However, discarding the transform coefficients corresponding to the highest frequencies from the representation produces small errors in image reconstruction.

Once the PCA and DCT coefficients of the decompositions are calculated, we propose three different methods for generating new images. In the first method, every component of the feature vector is randomly set to zero with a given probability. Then, the inverse of the transform is performed on the new feature vector, and a new image is generated. In the second method, some of the features at a random value extracted from a Gaussian distribution are reset. After that, the inverse of the transform is performed. In the third method, five random images in the dataset are selected that have the same label as a given image. We then perform a feature transform on all six images and to obtain their feature vectors. At this point, we randomly exchange some of features of the original image with some of the corresponding features of the five randomly selected images. We then perform the inverse of the transform to generate the new image, which is a mixture of the
six images, and label it the same as the others it was generated from. We do this for each image of the training set.

Figure 1 provides the pseudocode for each of the new DA methods. For the sake of space, we report the pseudocode for DCT only. The methods based on PCA are the same, except that the PCA space is built using the training data. The images generated by PCA and DCT are also reflected in the left-right direction with 50% probability for a further data augmentation.

The formula for calculating DCT used in the three algorithms presented in Figure 1 is the following:

\[
DCT_{image}(x, y) = \sum_{p,q=1}^{n} a_p a_q Image(p, q) \cos \frac{2p-1}{2n} \cos \frac{2q-1}{2n}
\]

where \( a_p = \begin{cases} 
\sqrt{\frac{1}{n}}, & n = 1 \\
\sqrt{\frac{2}{n}}, & n > 1
\end{cases} \) 

Algorithm MethodOne

**Input:** Image: tensor \( n \times n \times 3 \)  
**Output:** NewImage: tensor \( n \times n \times 3 \)  
channel <- 1;  
for every channel do  
  #DCTimage is a matrix of dimension \( n \times n \)  
  DCTimage <- calculateDCT(Image(:, :, channel)); # see Eq. 1  
  for row,col in DCTimage do  
    with probability 0.5 do  
      DCTimage(row, col) = 0; #except DCTimage(1,1) that cannot be reset  
    end  
  end  
  #inverse of the perturbated image  
  NewImage(:, :, channel) <- inverseDCT(DCTimage);  
end

Algorithm MethodTwo

**Input:** Image, tensor \( n \times n \times 3 \)  
**Output:** NewImage, tensor \( n \times n \times 3 \)  
channel <- 1;  
for every channel do  
  #DCTimage is a matrix of dimension \( n \times n \)  
  DCTimage <- calculateDCT(Image(:, :, channel)); # see Eq. 1  
  Sigma = standardDeviation(Image)/2;  
  for row,col in DCTimage do  
    #perturbation  
    DCTimage(row, col) = DCTimage(row, col) + sigma*randomNormal();  
  end  
end
Algorithm MethodThree

Input: Image: tensor $n \times n \times 3$
Images: list of $n \times n \times 3$ tensors
Output: tensor NewImage, $n \times n \times 3$
sample1,…,sample5 <- random images in Images whose label is the same of image
channel <- 1;
for every channel do
  #DCTimage is a matrix of dimension $n \times n$
  DCTimage <- calculateDCT(Image(:, :, channel)); # see Eq. 1
  for sample = sample1,…,sample5 do
    sampleDCT = calculateDCT(sample(:, :, channel));
    for row,col in DCTimage do
      with probability 0.05 do
        DCTimage(row, col) = sampleDCT(row,col);
      end
    end
  end
  #inverse of the perturbated image
  NewImage(:, :, channel) <- inverseDCT(DCTimage);
end

Figure 1. Pseudocode of the three DCT-based data augmentation approaches.

3 Experimental Results

3.1 Datasets

Several datasets that include very different image types were selected to test our system and
demonstrate the generalizability of our GenP bioimage system. Following a brief description of
each dataset is a descriptive summary of each dataset (see Table 2) that lists the number of classes
(#C), sample size (#S), image dimensions, and the URL for downloading the dataset. So that other
researchers can compare the results of their systems with the system proposed here, the datasets
used in our experiments are all publicly available:
• CH: the CHINESE HAMSTER OVARY CELLS [59] dataset of 327 fluorescent microscopy images that are divided into 5 classes;

• HE: the 2D HELA dataset [59] of 862 images of HeLa cells acquired by fluorescence microscope and divided into 10 classes.

• LO: the LOCATE ENDOGENOUS [60] dataset of 502 images of mouse sub-cellular images showing endogenous proteins or specific organelle features. The images are unevenly divided into 10 classes.

• TR: the LOCATE TRANSFECTED dataset of 553 mouse sub-cellular images showing fluorescence-tagged or epitope-tagged proteins transiently expressed in specific organelles [60]. The images are unevenly divided into 11 classes.

• RN: the FLY CELL dataset [60] of 200 images of fly cells acquired by fluorescence microscopy and divided into 10 classes.

• MA: Muscle aging [61] dataset of images of C. elegans muscles at 4 ages.

• TB: Terminal bulb aging [61] dataset of images of C. elegans terminal bulb at 7 ages (hence, 7 classes).

• LY: Lymphoma [61] dataset of malignant lymphoma of three subtypes.

• LG: Liver gender [61] dataset of liver tissue sections from 6-month male and female mice on a caloric restriction diet (the classes are the 2 genders).

• LA: Liver aging [61] dataset of liver tissue sections from female mice on ad-libitum diet of 4 ages.

• CO: Collection of textures in histological images of human colorectal cancer [62].
TABLE 2. DESCRIPTIVE SUMMARY OF THE DATASETS

| Dataset | #C | #S   | Size       | URL for Download                        |
|---------|----|------|------------|-----------------------------------------|
| CH      | 5  | 327  | 512×382    | http://ome.grc.nia.nih.gov/iicbu2008/hela/index.html#cho |
| HE      | 10 | 862  | 512×382    | http://ome.grc.nia.nih.gov/iicbu2008/hela/index.html     |
| LO      | 10 | 502  | 768×512    | http://locate.imb.uq.edu.au/downloads.shtml           |
| TR      | 11 | 553  | 768×512    | http://locate.imb.uq.edu.au/downloads.shtml           |
| RN      | 10 | 200  | 1024×1024  | http://ome.grc.nia.nih.gov/iicbu2008/mai/index.html    |
| TB      | 7  | 970  | 300×300    | https://ome.grc.nia.nih.gov/iicbu2008                   |
| LY      | 3  | 375  | 1388×1040  | https://ome.grc.nia.nih.gov/iicbu2008                   |
| MA      | 4  | 237  | 1600×1200  | https://ome.grc.nia.nih.gov/iicbu2008                   |
| LG      | 2  | 265  | 1388×1040  | https://ome.grc.nia.nih.gov/iicbu2008                   |
| LA      | 4  | 529  | 1388×1040  | https://ome.grc.nia.nih.gov/iicbu2008                   |
| CO      | 8  | 5000 | 150×150    | https://zenodo.org/record/53169#.WaXjW8hJaUm           |

Unless specified otherwise in the description of the dataset above, the protocol used in our experiments was the five-fold cross-validation method. Also, to avoid overfitting, the same set of descriptor parameters were used for all descriptors across all tested datasets. Each of the experiments reported here were statistically validated using the Wilcoxon signed rank test [63].

3.2 Experiments

In Table 3 we report the performance obtained by some of the handcrafted features and the following ensembles:

- FH: sum rule among LTP, MLPQ, CLBP, RICLBP, LET, MOR, AHP, FBSIF, COL (on only the datasets with colored bioimages) and ETAS;
- FH-etas: same as FH but without considering ETAS;
- FUS1: sum rule of FH and CLM
- FUS2: sum rule of FUS1 and GOLD.

Before each fusion, the scores of the SVMs trained with a given descriptor are normalized to mean 0 and standard deviation 1.

In the last row of Table 3, labelled OLD, we report the performance of the handcrafted ensembles tested in [50]. In the column labeled Average, we report the average accuracy obtained by a given descriptor/ensemble across the entire set of tested datasets.

Examining Table 3 we find that FBSIF and MLPQ obtained the best performances among the tested individual descriptors. There is no statistical difference between these two methods, however; both outperform all the other individually tested methods with a p-value of 0.1. The best performing ensembles are FUS1 and FUS2. They outperform the other ensembles, as well as the best performing individual descriptors, FBSIF and MLPQ, with a p-value of 0.1.

In the last set of experiments, we compare the results of the deep learning features with the other features. We also propose a mixed-type ensemble that we compare with several state-of-the-art methods. Because FUS1 and FUS2 produced similar results, we use FUS1 (because it’s simpler than FUS2) in the following tests.
Table 3. Performance of Local-Based Approaches and their Fusion

|       | CH   | HE   | LO   | TR   | RN   | TB   | LY   | MA   | LG   | LA   | CO   | Avg  |
|-------|------|------|------|------|------|------|------|------|------|------|------|------|
| LTP   | 98.77| 87.33| 94.6 | 90.55| 80   | 55.88| 83.33| 73.75| 92.00| 98.67| 90.40| 87.11|
| MLPQ  | 99.38| 92.79| 97.6 | 97.09| 88.5 | 62.89| 92.27| 91.67| 99.33| 99.81| 93.88| 92.26|
| CLBP  | 94.15| 89.42| 86.2 | 84   | 70   | 61.03| 86.67| 75.42| 96.00| 99.24| 92.04| 84.92|
| RICLB | 96.62| 85.35| 92.6 | 91.82| 82   | 54.54| 85.87| 91.67| 99.33| 99.62| 91.56| 88.27|
| LET   | 97.85| 92.33| 95.80| 92.91| 75.00| 54.85| 92.53| **98.75**| **100**| 99.81| 93.18| 90.27|
| MOR   | 97.85| 84.88| 93.60| 92.36| 83.50| 56.60| 84.53| 80.00| 96.33| 98.29| 93.30| 87.38|
| GOLD  | 92.62| 85.81| 87.8 | 75.45| 50   | 55.05| 53.07| 66.67| 85.00| 39.24| 83.58| 70.39|
| AHP   | 98.77| 91.86| 96.4 | 95.45| 88   | 59.48| 93.87| 90.42| 98.67| 99.81| 94.16| 91.53|
| FBSIF | 99.38| 94.19| 98.2 | 98.55| 87   | 65.67| 92.53| 88.75| 100   | 99.81| 93.42| 92.50|
| COL   | ---  | ---  | ---  | ---  | ---  | ---  | 91.47| ---  | 99.67| 99.62| 92.30| ---  |
| ETAS  | 84.92| 73.95| 95.00| 84.91| 59.50| 51.03| 87.73| 69.58| 98   | 98.29| 92.04| 81.35|
| CLM   | 98.15| 91.05| 95.40| 90.73| 82.00| 68.56| 74.40| 91.67| 99.67| 96.95| 89.60| 88.92|
| FH    | 99.69| 93.95| 98.60| 98.55| 91.00| 68.35| 94.67| 92.08| **100**| **100**| **95.20**| 93.82|
| FH-etas| 99.69| 93.95| 98.20| 98.55| 90.50| 68.04| 94.13| 91.67| **100**| **100**| 95.18| 93.62|
| FUS1  | **100**| 94.88| **98.80**| **98.91**| **92.00**| 71.24| **94.67**| 92.50| **100**| **100**| 95.18| 94.38|
| FUS2  | **100**| **95.70**| **98.80**| **98.36**| **92.00**| **71.86**| **93.87**| **93.33**| **100**| **100**| 94.94| **94.44**|
| OLD   | 99.69| 94.42| 98.40| 98.36| 90.50| 70.62| 92.00| 91.67| **100**| 99.62| 93.74| 93.54|

In Table 4 we compare the different approaches for data augmentation, reporting the performances using ResNet50 and DenseNet. The method labelled ENS is the sum rule among the CNNs trained using the six data augmentation approaches. The performance reported in Table 3 is the sum rule of each method trained with the two different learning rates and the two different batch sizes.
Clearly, the best approach is given by ENS, which outperforms all the other approaches with a p-value of 0.01. Among the stand-alone approaches, the best performance is obtained by the DCT-based method. These results demonstrate the value in using feature transforms for enlarging datasets and improving the performance of CNN.

Finally, in table 5 we compare the performance of some of our ensembles with several state-of-the-art approaches reported in the literature.

The following ensembles are reported in Table 5:

- **HANN**: the method named FUS1 in Table 3;
- **DEEP**: ensemble of all the trained CNNs (using different the different values for LR, BS, and DA);
- **DEEP(1-4):** the same as DEEP but using only four CNNs (AlexNet; GoogleNet, Vgg16, and Vgg19);

- **HAND+DEEP:** sum rule between HAND and DEEP.

- **HAND+ENS Dense:** sum rule between HAND and the ensemble of the different DenseNet.

When we combine two methods by sum rule, their scores are normalized before the fusion to mean 0 and standard deviation 1.

**Table 5. Comparison with the state of the art**

|        | CH  | HE  | LO  | TR  | RN  | TB  | LY  | MA  | LG  | LA  | CO  |
|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Hand   | **100.00** | 94.88 | 98.80 | 98.91 | 92.00 | 71.24 | 94.67 | 92.50 | **100.00** | **100.00** | 95.18 |
| Deep   | 99.38 | 96.51 | **99.20** | 98.55 | 86.50 | 74.64 | 92.80 | 98.33 | **100.00** | 99.24 | **97.40** |
| Deep(1-4) | 99.38 | 95.70 | 99.00 | 98.55 | 79.50 | 73.20 | 89.87 | 95.00 | **100.00** | 98.86 | 96.78 |
| Hand+Deep | **100.00** | 97.21 | **99.20** | **99.09** | **93.50** | 75.67 | **96.87** | **98.75** | **100.00** | **100.00** | 97.00 |
| Hand+EnsDen | **100.00** | 96.51 | 98.80 | **99.09** | 93.00 | **75.88** | 96.27 | 97.92 | **100.00** | **100.00** | **97.32** |

[50] 100.00 95.93 98.60 98.55 91.50 75.15 90.67 94.58 100.00 100.00 93.98

[64] 99.90 **98.30** -- -- 86.50 64.80 96.80 97.90 99.60 **100.00** --

[65] 98.50 94.4 95.60 88.10 67.50 44.60 -- -- -- -- --

[66] 93.00 84.00 -- -- 82.00 49.00 85.00 53.00 99.00 51.00 --

[3] 93.10 68.30 -- -- 55.00 51.10 70.90 89.60 91.70 73.8 --

[7] 99.00 84.00 -- -- 73.00 55.00 66.00 -- 99.00 89.00 --

[67] 98.40 90.70 -- -- 90.10 -- -- -- -- --

[68] -- -- -- -- -- -- -- 92.7 -- 99.20 96.40 --

[62] -- -- -- -- -- -- -- -- -- -- --

[69] -- 93.08 -- -- -- -- -- -- --

[70] -- 89.37 -- -- -- -- -- -- --
**HAND + DEEP** is the best ensemble proposed here: it outperforms all the other approaches reported in the experimental section with a p-value of 0.1. Clearly the proposed ensemble outperforms the ensembles in [50] and [64].

Unlike the other state-of-the-art methods, the full MATLAB source code for reproducing results is freely available. Given that all the descriptors can be calculated in parallel by exploiting the modern multicore CPUs (for handcrafted features) and GPUs (for deep learning features), all the descriptors can be extracted in a reasonable amount of time for all applications where real-time computation is not important (which is the case for many medical image classification problems).

### 4 Conclusion

In this paper we propose a GenP bioimage ensemble that combines multiple handcrafted and learned texture descriptors. An ensemble of deep learning methods is built using different criteria (different batch sizes, learning rates, topologies, and methods of data augmentation). We also propose three new methods for data augmentation based on feature transforms (principal component analysis and discrete cosine transform) that boost the performance of Convolutional Neural Networks (CNNs). Each handcrafted descriptor is used to train a different Support Vector Machine (SVM), and the different SVMs are combined with the ensemble of CNNs. The experimental section shows that a boost in performance is obtained by combining local features, dense sampling features, and deep learning approaches using augmented images. The discriminative power and generalizability of our best performing bioimage system, DeepHand, is verified on a wide range of publicly available bioimage benchmark datasets, each of which represents different bioimage classification tasks.

The main contributions of the proposed paper are the following:

- The proposal of three new approaches for data augmentation based on PCA/DCT;
• The demonstration that different data augmentation approaches can be used for building an ensemble of CNNs;

• The proposal of a set of handcrafted/learned descriptors that is not only highly generalizable but that also obtains state-of-the-art performance on a large set of datasets.

In the future we plan on exploring methods for combining this system with other dense patch approaches, such as IFV. We also plan on investigating methods for training CNNs on smaller training sets and for reducing the dimensions of deeper CNN layers.

To reproduce the experiments reported in this paper, the MATLAB code of all the descriptors is available at https://github.com/LorisNanni.

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