

Local focus-tolerant image descriptors for classification of biological particles

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Abstract—In this work we present a new approach to the extraction of features robust to focal mismatches, for the classification of biological particles characterized by 3 dimensional structures. We use SIFT descriptors in order to encode local gradient, fused with features derived from an introduced adaptive filterbank of Gabor filters. We have evaluated the proposed technique using a dataset consisting of 174 images of pollen grains from 29 species, acquired with a low-cost optical microscope in arbitrary focal planes. The proposed descriptor efficiently captures discriminative information by encoding the local inner and outer structure of the transparent pollens in a focus-tolerant manner, achieving approximately 74.5% classification accuracy, demonstrating that local scale invariant features can be robust even under challenging conditions.

I. INTRODUCTION

THE science of Palynology has nowadays exceeded the boundaries of Paleopalynology and has become one of the most diverse sectors that use biological particles. Palynological data are currently used in various applications such as Allergy research, Aeropalynology, Forensic and food science. Pollen identification, dispersion and pollen grain concentration are the most important topics encountered in all applications. The procedure of taxonomic classification of the different pollens is a very time consuming and laborious work and is until today mainly held by highly skilled experts using microscopic equipment. In order to increase reproducibility of the results and achieve identification specificity with reduced effort, developing an automated pollen classification system can be a valuable tool for scientists.

The last two decades several automated classification systems have been proposed utilizing pollen images acquired from microscopic devices [1, 2, 3, 4]. Due to the different applications, data types and the complexity of the problem considered, a variety of methods have been presented. In most cases, private pollen image databases have been used, stemming from specific geographical areas and containing

representative local plant taxa.

It is common in many studies to use a combination of features in order to improve the classification performance. Texture, shape and color features are the most important information due to the nature of the data. In a recent study, Chica et al. [5] aimed to identify five local pollen types against fraudulent samples (outliers), using a multi-classifier model based on discriminative surface characteristics such as shape, texture and color information. This method is focal plane depended, requesting the user to provide as input three specific focal images for each pollen grain. A combination of color features over decorrelated stretched images along with standard characteristics like Fourier descriptors and geometrical features was introduced in [6]. Having available 426 pollen grain images corresponding to 17 genders and species, multiple instances on different preprocessed images are required for the classification. Earlier, Rodriguez-Damian et al. [7] proposed a method based on brightness and shape descriptors for recognizing species of the Urticaceae family, achieving their best classification performance using Fourier descriptors. In a series of studies [8, 9, 10] from the Massey University of New Zealand introduced combinations of surface texture features along with shape features to address the problem of automated pollen analysis. Allen et al. [11], based on [10] developed a complete system (microscope and image processing software) for pollen detection and classification. Although achieving high performance, the system requires specific focal settings. Dahme et al. [2] modeled the problem via texture features derived from a specific filterbank, including additional spatial distributions of textons. Chen et al. [3] investigated the feasibility of a classification system targeted to airborne pollen. In this work, the best discriminative classification performance was achieved using simple geometrical features including morphological features such as Fourier descriptor component for mugwort colpi. In [4] Nguyen et al. introduced among other improvements, a new descriptor of the pollen spikes as well as a new selection criterion to obtain the most confusing samples of the target types from the unlabeled data, applied in a database of 768 grains from 9 pollen types. In the framework of the European project ASTHMA, Boucher et al. [12] approached the problem of pollen recognition using multifocal (with a predefined step) color images, of four pollen types, automatically selected. There are also studies that approach the problem through searching for geometrical and/or radial deformation invariants, such as in [13], achieving a very good classification accuracy, using though confocal microscopy or

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3D volumetric data.

In this work, we propose a method for classification of single-grain images captured via microscopy, without considering any specific acquisition conditions such as focal and view planes. These properties can be useful in situations where there is lack of metadata regarding the acquisition conditions of the images, or low-cost microscopic equipment is used where accurate positioning of the focal plane is difficult. Towards this purpose, we utilize a scale-invariant key-points detector for the extraction of rotation-invariant features that exhibit robustness to focus mismatches. Finally, developing an automated classification system for multi-focus images can be valuable in a variety of biomedical and biological applications (it could also contribute to the laborious work of organizing the old datasets).

This paper is organized as follows: in Section 2 there is a brief description of pollen grain characteristics and the taxonomy used in this work. The proposed feature extraction procedure is detailed in Section 3, and the classification algorithm in Section 4. Finally, experimental results are given in Section 5 and conclusions are drawn in Section 6.

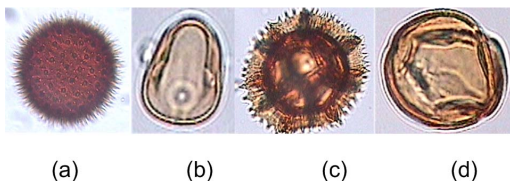


Fig. 1. Inter class variations. Four different pollen grain images, each typical for its species: a) *Lavatera cretica*, b) *Echium plantagineum*, c) *Carthamus lanatus* and d) *Buxus sempreviverens*

II. POLLEN CHARACTERISTICS - TAXONOMY

In nature, pollen grains are found as 3D complex structures that carry the plants' male DNA in order to achieve pollination, being thus an integral part of the circle of life. These important biological particles exhibit great diversity in sizes, shapes, colors and texture patterns on their cell wall, which are characteristic for each taxon or species. More specifically the size ranges between $10\mu\text{m}$ to $200\mu\text{m}$ and the surface can present various ornamentations and can vary from smooth to spiky. Pollen also usually bare a number of colpi and/or germination pores and are also characterized by their polarity and symmetry. Pollen morphology varies for each plant and is likely to be diverse even within a species. Each grain type has a unique set of features that makes each pollen type unique (Figure 1).

Therefore, by exploiting their characteristic structure their origin can be determined even at species level. For pollen recognition three classification criteria are most commonly used by experts: 1) the size, 2) the number and location of pori and/or colpi, and 3) the detailed structure of the cell wall features. Shape is also another classification criterion, although it cannot be considered to be a reliable feature, as it may differentiate due to environmental and/or preparation factors.

Pollen grains are observed and analyzed via microscopy.

In the related literature (i.e.: [9, 10, 2]) pollen images are usually acquired from optical microscope (LM) or scanning electron microscope (SEM), with the second being less used due to its high cost. Although they are actually three dimensional particles, this is difficult to be seen under the most common microscopes. Hence, they are described in as two dimensional images, in polar or/and equatorial view. Depending on the focal depth, the sequence of images received for each pollen grain varies, as it presents a different layer achieving a view in different characteristics of the grain. Small changes in focus can result great variations in the pollen's visual features such as texture. In this work we consider the problem where the images of each pollen grain are derived from different focal depths, with no specific focal step.

Each country has each distinct flora map consisting of vast number of pollen types and sometimes including unique species. Nevertheless, each wider geographical region has common pollen types. In our method, pollen from typical species of the Mediterranean basin were used; namely: *Anemone pavonina*, *Bellis perennis*, *Buxus sempreviverens*, *Carthamus lanatus*, *Cerinth major*, *Echium italicum*, *Echium plantagineum*, *Genista canthoclada*, *Gladiolus italicus*, *Lavatera cretica*, *Ligustrum lucidum*, *Lomelosia branchiata*, *Matricaria chamomilla*, *Medicago coronata*, *Micromeria juliana*, *Ononis viscosa*, *Oxalis pes caprae*, *Pallenis spinosa*, *Papaver rhoeas*, *Plantago lagopus*, *Reseda lutea*, *Silene colorata*, *Sinapis alba*, *Solanum eleagnifolium*, *Sorghum halepense*, *Spartium junceum*, *Tordylium apulum*, *Trigonella corniculata*, *Vitex agnus castus*.



Fig. 2. Intra-class texture variation due to the different focal plane (species depicted here: *Anemone pavonina*).

III. FEATURES EXTRACTION

As stated in the introduction, in this work we propose a system for automatic classification of pollen-grain images, without considering specific information regarding the focal plane and view of the depicted grains. Such an approach is very challenging due to the nature of the grains, where small variations of the focal plane can result drastic changes of visual characteristics such as texture and shape in the acquired images, as can easily be seen in Figure 2.

Thus, we need a mechanism able to extract features insensitive to lens blurring, which at the same time can efficiently encode discriminative optical characteristics of the grains. To this purpose we have incorporated the key-points selection algorithm proposed by D. Lowe [14] as a detector of image locations where scale-invariant characteristics are presented. We argue that such points are the most suitable for extracting features which are robust to focal mismatches, since the effect of lens blurring can be

approximated by a Gaussian blurring for small displacements of the focal plane. Hence, the mechanism used by Lowe’s algorithm where the candidate points are selected based on the strong response to Difference-of-Gaussians (DoG) filtering of the image which emulates the different scales, is a good approximation of seeking for points where focus-invariant features can be extracted (for a detailed description of the algorithm refer to [14]).

Following the key-points detection procedure, a set of features is extracted at the corresponding image locations, aiming to encode the discriminative structural characteristics of the pollen grains. To this purpose, the SIFT descriptors [14] are incorporated in order to encode local gradient information which can efficiently describe visual structures like spikes, pores, colpi etc. The SIFT descriptor consists of the concatenated gradient orientation histograms computed on a region of 4×4 rectangular sub-neighborhoods of radius r around a key-point. The radius is dictated by the previously estimated optimal scale for feature extraction, and the reference orientation used in the calculation of orientation histograms is aligned to the dominant gradient orientation of the neighborhood. Therefore, SIFT features can offer a rotation and scale invariant description of visual information around the key points (for further details refer to [14]).

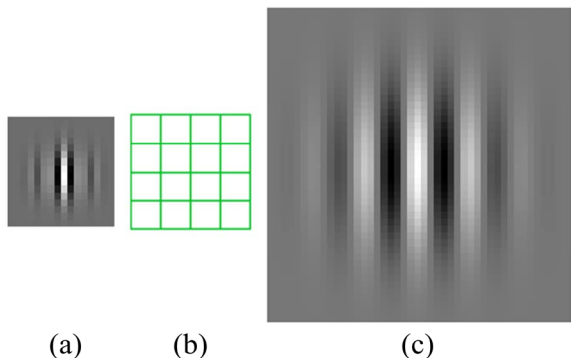


Fig. 3. The real part of the impulse response corresponding to the reference Gabor filter bank (a) at the smallest scale and (c) at the largest scale, in comparison to the $r=2$ SIFT neighborhood.

Additionally, we introduce an adaptive variation of Gabor filters in order to perform local spectral decomposition of the image on the surrounding regions of key-points. This type of information can efficiently encode both structural and textural characteristics of the depicted grains, as Gabor are known to exhibit optimal behavior on localizing the spectral information due to their minimum space-frequency uncertainty. A Gabor filter bank consisting of filters in 4 scales and 6 orientations, designed for optimal cover of the spectral domain [15], is used as the reference bank. Towards constituting the extracted features invariant to rotation and scale, the reference filter bank is rotated in order to be aligned to the dominant gradient direction around each key-point, and the scale is adjusted proportionally to the radius r of the SIFT descriptors. The reference filter bank contains filters with center frequency between 0.11 cycles/pixel and

TABLE I
EXPERIMENTAL RESULTS

Method	Classification Accuracy (%)
Gabor (<i>Mean</i>)	53.3
Gabor (<i>Mean & Skewness</i>)	56.3
<i>SIFT</i>	70.0
<i>SIFT & Adaptive Gabor</i>	74.5

0.3 cycle/pixel, and is matched to a region corresponding to $r=2$ pixels, as illustrated Figure 3.

At each key-point of a given image, the SIFT descriptor is calculated producing a 128-dimensional feature vector $\mathbf{F}^S \in \mathbb{R}^{128}$, and the adjusted Gabor filters are convolved with the image patch corresponding to the region covered by the SIFT descriptor. The magnitude of each filter’s output averaged in the whole region constitutes one feature of the 24-dimensional Gabor feature vector $\mathbf{F}^G \in \mathbb{R}^{24}$. Finally, the two descriptors are concatenated in order to form the final 152-dimensional descriptor $\mathbf{F} = [\mathbf{F}^S \mathbf{F}^G]^T \in \mathbb{R}^{152}$ of the key-point.

IV. CLASSIFICATION

Given a pollen image i , a set of features $D_i = \{\mathbf{F}_1^i, \mathbf{F}_2^i, \dots, \mathbf{F}_N^i\}$ is computed on the detected N key-points. We define the similarity between two images i and j as the number of matched key-points between the two images. A key-point k of the image i is considered to be matched to the key-point l of the image j , if the feature vector \mathbf{F}_l^j is the closest to the vector \mathbf{F}_k^i among the features set D_j calculated using the L_1 norm, and the corresponding distance is smaller than a threshold τ . More formally, the similarity $S(i, j)$ is defined as follows:

$$S(i, j) = \sum_{k=1}^{N_i} \sum_{l=1}^{N_j} \text{match}(k, l),$$

$$\text{match}(k, l) = \begin{cases} 1 & \text{if } \min_{l \in \{1, \dots, N_j\}} (\|\mathbf{F}_k^i - \mathbf{F}_l^j\|_1) < \tau \\ 0 & \text{else} \end{cases}$$

Using the above definition of similarity between two images and a labeled training, a test image can be easily classified using the Nearest Neighbor rule, with the class-label of the most similar image of the training set. Formally,

$$\text{Class}(i) = \text{label} \left(\arg \max_j \{S(i, j)\} \right).$$

V. EXPERIMENTAL EVALUATION

For the evaluation of the proposed method, we used a database consisting of 5 single-grain images from each of

the 29 species listed in Section 2. The images were acquired using an optical microscope, using the procedure described in [16]. The view and focal plane of each image is arbitrary, and the only preprocessing followed is the conversion to gray-scale.

Due to the small number of samples we followed the Leave-One-Out experimental protocol, where 29 images (one randomly chosen image from each species) constitute the test set, and the rest 116 images form the training set. Twenty iterations of the experiment were performed, performing a new randomization of the training and test set on each one, and the results were averaged. The threshold was determined experimentally to $\tau=136$ by performing cross-validation on a different set of images.

To gain some insights regarding the contribution of each individual component of the proposed scheme, we have conducted additional experiments under the same protocol using only the SIFT features as image descriptors. Additionally, we have evaluated the reference Gabor filter bank as a typical texture descriptor, by convolving each filter along the whole image area, and computing the first four statistical moments of the output magnitude of each filter. The obtained results are summarized in Table I.

It is apparent that global texture is the less discriminative information under such arbitrary conditions, due to reasons previously explained. The SIFT descriptors are much more efficient into capturing discriminative information achieving 70% classification accuracy. Finally, the fusion of SIFT and Local Adaptive Gabor features are the most efficient, achieving 74.48% accuracy. These results strengthen our argument that local scale-invariant features can be robust to image distortions caused by focal mismatches, and are able to encode discriminative information even under very challenging conditions. Furthermore, the proposed Local Adaptive Gabor features can capture complementary information to gradient descriptors like SIFT, even under conditions where texture is not a reliable visual characteristic for discrimination.

VI. CONCLUSIONS

In this study we presented the use of a novel variation of Gabor filters that are adapted to the estimated optimal scale for the feature extraction in order to acquire features that efficiently represent local structural and textural characteristics of biological particles like pollen grains, as a means to enhance the information acquired from gradient descriptors such as SIFT. We evaluated our algorithm to the task of pollen grain recognition from images where no specifications regarding the focal plane and the view of the depicted pollen-grain are considered. We achieved accuracy as high as 74.5% by fusing SIFT and local adaptive Gabor descriptors. Considering the nature of the problem we have addressed to, we have shown that in such challenging visual conditions, local scale-invariant features can effectively encode discriminative information hence exhibiting robustness to image distortions.

REFERENCES

- [1] Norma M. García, V. A. E. Chaves, J. C. Briceño, C. M. Travieso, "Pollen Grains Contour Analysis on Verification Approach," in *Proc. 7th International Conference, HAIS, Spain, 2012*, pp. 521-532.
- [2] Dahme, Gary, Eraldo Ribeiro, and Mark Bush. "Spatial statistics of textons," *International Conference on Computer Vision Theory and Applications*, 2006, pp. 13-19.
- [3] Chun Chen, Emile, A. Hendriks, Robert P. W. Duin, Johan H. C. Reiber, Pieter S. Hiemstra, Letty A. de Weger, Berend C. Stoel, "Feasibility study on automated recognition of allergenic pollen: grass, birch and mugwort," *Aerobiologia*, 2006, Vol. 22, pp 275-284.
- [4] Nhat Rich Nguyen, Matina Donalson-Matasci, Min C. Shin, "Improving pollen classification with less training effort," *IEEE Workshop on Applications of Computer Vision (WACV)*, 2013, pp.421-426.
- [5] Chica M, "Authentication of bee pollen grains in bright-field microscopy by combining one-class classification techniques and image processing," *Microsc Res Tech.* (11), 2012, pp. 1475-85.
- [6] Jaime R. Ticay-Rivas, Marcos del Pozo-Baños, Carlos M. Travieso, Jorge Arroyo-Hernández, Santiago T. Pérez, Jesús B. Alonso, Federico Mora-Mora, "Pollen Classification Based on Geometrical, Descriptors and Colour Features Using Decorrelation Stretching Method," in *Proc. 12th INNS EANN-SIG International Conference, EANN and 7th IFIP WG 12.5 International Conference, AIAI, Greece, 2011*, pp. 342-349.
- [7] Rodríguez-Damian M., Cernadas, E., Formella, A., Sa-Otero, R., "Pollen classification using brightness-based and shape-based descriptors," in *Proc. of the 17th International Conference on Pattern Recognition, ICPR 2004., vol.2, no., pp.212,215 Vol.2, 23-26, 2004.*
- [8] W. J., Taylor G. E. and Flenley J. R., 2004 "Towards automation of palynology 1: analysis of pollen shape and ornamentation using simple geometric measures, derived from scanning electron microscope images," *J. Quaternary Sci.*, Vol. 19, pp. 745-754.
- [9] Li, P. Treloar, W. J. Flenley, J. R. and Empson, L. 2004. "Towards automation of palynology 2: the use of texture measures and neural network analysis for automated identification of optical images of pollen grains," *J. Quaternary Sci.*, Vol. 19, pp. 755-762.
- [10] Zhang, Y., Fountain, D. W., Hodgson, R. M., Flenley, J. R. and Gunetileke, S. "Towards automation of palynology 3: pollen pattern recognition using Gabor transforms and digital moments," *J. Quaternary Sci.*, Vol.19, 2004, pp. 763-768.
- [11] G. Allen, B. Hodgson, S. Marsland, G. Arnold, R. Flemmer, J. Flenley, and D. Fountain, "Automatic recognition of light microscope pollen images," 2006.
- [12] Al. Boucher, Pablo J. Hidalgo, M. Thonnat, J. Belmonte, C.Galan, P.Bonton, R.Tomczak, "Development of a semi-automatic system for pollen recognition," *Aerobiologia*, Vol. 18, 2002, pp 195-201.
- [13] Olaf Ronneberger, Qing Wang, Hans Burkhardt, "3D Invariants with High Robustness to Local Deformations for Automated Pollen Recognition," in *Proc., 29th DAGM Symposium, Germany, 2007*, pp 425-435.
- [14] David G. Lowe, "Distinctive image features from scale-invariant keypoints," *International Journal of Computer Vision*, 2004, 60, pp. 91-110.
- [15] B.S Manjunath, W.Y Ma, "Texture features for browsing and retrieval of image data," *IEEE Trans. Pattern Analysis and Machine Intelligence*, vol.18, pp.837, 842, Aug 1996.
- [16] M. Koutoula, "Contribution in creation of a palynologic atlas of the flora of the University city of Patras," M.S. thesis, Dept. Biology, Patras Univ., Patras, Greece, 2004.