

Automated pollen identification using microscopic imaging and texture analysis

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Abstract

Pollen identification is required in different scenarios such as prevention of allergic reactions, climate analysis or apiculture. However, it is a time-consuming task since experts are required to recognize each pollen grain through the microscope. In this study, we performed an exhaustive assessment on the utility of texture analysis for automated characterization of pollen samples. A database composed of 1800 brightfield microscopy images of pollen grains from 15 different taxa was used for this purpose. A pattern

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recognition-based methodology was adopted to perform pollen classification. Four different methods were evaluated for texture feature extraction from the pollen image: Haralick's Gray-Level Co-occurrence Matrices (GLCM), Log-Gabor Filters (LGF), Local Binary Patterns (LBP) and Discrete Tchebichef Moments (DTM). Fisher's discriminant analysis and k -nearest neighbour were subsequently applied to perform dimensionality reduction and multivariate classification, respectively. Our results reveal that LGF and DTM, which are based on the spectral properties of the image, outperformed GLCM and LBP in the proposed classification problem. Furthermore, we found that the combination of all the texture features resulted in the highest performance, yielding an accuracy of 94.83%. Therefore, thorough texture characterization could be considered in further implementations of automatic pollen recognition systems based on image processing techniques.

Keywords: Texture analysis, Pollen identification, Gray-Level Co-occurrence Matrix, Log-Gabor Filters, Local Binary Patterns, Discrete Tchebichef Moments

1. Introduction

Palynology is the study of pollen grains produced by seed plants and spores (Erdtman et al., 1969). Accurate identification of pollen types is a relevant issue in several scenarios. For instance, quantifying the concentration of airborne pollen may help people suffering from allergic reactions to adopt adequate treatment strategies. In archaeology, pollen fossils are analyzed to reconstruct ecological and climate conditions during past periods. For commercial purposes, pollen is involved in the search of oil and gas. In apiculture, pollen classification is required to identify nectar sources, which determine the quality of the product and enable the authentication of its origin (Kaya et al., 2013). Currently, pollen identification is based on visual inspection of microscopy images. It is a time-consuming and costly procedure since a trained expert must manually classify each pollen grain (Mitsumoto et al., 2009). Furthermore, a subjective result is obtained as it depends on the expert’s criterion. Thus, automated methods for pollen identification are required in order to overcome the limitations of the conventional procedure (Stillman and Flenley, 1996).

Slides observed through the microscope contain a variable number of pollen grains. Therefore, automatic pollen identification would involve segmentation and classification tasks (Kumar et al., 2010). Segmentation aims to localize each of the pollen grains in the slide, separating it from the rest of the content. In classification, the isolated pollen grain is assigned to one of a predefined set of categories (taxa). The present study is focused on the latter. For this purpose, brightfield microscopy images corresponding to a subset of honey-bee pollen taxa commonly found in the mediterranean area

26 were analyzed. Previous studies in the field of automated palynology focused
27 on this specific type of pollen samples (Chica, 2012; Carrión et al., 2004).

28 A pattern recognition approach was adopted to model the pollen grain
29 classification problem. Hence, it is assumed that grains from a given taxon
30 own distinctive characteristics with respect to samples from other taxa (Duda
31 et al., 2012). Pattern recognition mainly involves feature extraction and mul-
32 tivariate pattern classification (Bishop, 1995). The former consists in the
33 definition of a set of quantitative measurements, called features or descrip-
34 tors, capturing representative attributes of the image (pollen grain) to be
35 identified. As a result, the grain is described by a point in the multivari-
36 ate space defined by the feature set. Subsequently, a multivariate classifier
37 is then used to define decision boundaries between categories in the input
38 feature space.

39 Diverse features have been previously evaluated in automated classifica-
40 tion of pollen taxa based on image processing techniques. Morphological
41 and geometric descriptors have been the most common choice for this pur-
42 pose (Kaya et al., 2013; Mitsumoto et al., 2009; Boucher et al., 2002; Treloar
43 et al., 2004; Chica, 2012). They include measurements such as area, perime-
44 ter, concavity, convexity or circularity of the grain. Also, the utility of pore
45 detection has been evaluated (Chen et al., 2006). However, it was reported
46 that similar pore appearances can be found in samples from different taxa,
47 reducing the discriminative capability of this approach (Chen et al., 2006).
48 Additionally, texture attributes of the grain’s surface or exine have been
49 suggested as pollen descriptors. Pollen recognition from texture analysis is
50 based on the differences found between the ornamentation of the exines from

51 distinct taxa. Previously, texture descriptors provided promising results in
52 pollen identification both separately (Kumar et al., 2010; Langford et al.,
53 1990; Li and Flenley, 1999; Carrión et al., 2004; Li et al., 2004; Zhang et al.,
54 2005) and combined with morphological features (Chen et al., 2006; Zhang
55 et al., 2004; Rodriguez-Damian et al., 2006; Punyasena et al., 2012).

56 In particular, texture represents an efficient approach for pollen iden-
57 tification as it provides several advantages. As the rest of the techniques
58 based on image processing, texture methods could be integrated in a soft-
59 ware capable of automatically assessing large amounts of pollen samples. In
60 addition, texture analysis enables the inspection of broken grains typically
61 found in fossil pollen samples for which morphological descriptors would be
62 inappropriate. Furthermore, in contrast to methods based on pore detec-
63 tion, texture properties do not depend on the position of the grain in the
64 slide. Despite the benefits of the texture-based approach, a reduced number
65 of texture methods have been applied to pollen characterization. Commonly,
66 previous studies focused on pollen texture used conventional first-order and
67 second-order statistical features, with the latter being computed from Har-
68 alick’s co-occurrence analysis (Langford et al., 1990; Li et al., 2004; Chen
69 et al., 2006; Rodriguez-Damian et al., 2006). Occasionally, other techniques
70 included Laws masks (Li and Flenley, 1999; Carrión et al., 2004) and Gabor
71 filters (Zhang et al., 2004). On the other hand, a small number of taxa,
72 typically lower than 10, was analyzed in these preceding research works.
73 Therefore, further analysis including other different methods and a higher
74 variety of taxa is required to obtain solid conclusions about the potential of
75 texture in pollen identification.

76 The purpose of the present study is to perform an exhaustive analysis
 77 on the correspondence between the texture of a pollen grain and its taxon.
 78 Texture is a property related to the roughness of a surface and is charac-
 79 terized by the variation of pixel intensity in the spatial domain (Tuceryan
 80 and Jain, 1993). A number of texture descriptors have been proposed in the
 81 literature. Tuceryan and Jain (Tuceryan and Jain, 1993) grouped them into
 82 four types: statistical, geometrical, model-based and signal processing. In
 83 our study, we selected a subset of texture methods in order to capture dif-
 84 ferent properties of the pollen texture. Haralick’s Gray-Level Co-occurrence
 85 Matrices (GLCM) (Haralick et al., 1973) were used as a reference method
 86 in our experiments since it has been adopted by other researchers (Langford
 87 et al., 1990; Li et al., 2004; Chen et al., 2006; Rodriguez-Damian et al., 2006).
 88 In addition, we evaluated the utility of other techniques that have not been
 89 previously applied to texture-based pollen classification namely Log-Gabor
 90 Filters (LGF) (Field et al., 1987), Local Binary Patterns (LBP) (Ojala
 91 et al., 2002) and Discrete Tchebichef Moments (DTM) (Mukundan et al.,
 92 2001). These methods have provided satisfactory results in other texture and
 93 pattern recognition problems (Soh and Tsatsoulis, 1999; Gao et al., 2007;
 94 Das et al., 2013; Marcos and Cristóbal, 2013).

95 This paper is organized as follows. The second section describes the ac-
 96 quisition of pollen images as well as the database employed in our research.
 97 In the third section, the applied methodology is presented. An explanation
 98 of the techniques used for image segmentation and preprocessing, texture
 99 feature extraction, dimensionality reduction and pattern classification is pro-
 100 vided. The fourth section of the paper includes the results achieved in our

101 experiments as well as an analysis of them. Finally, in the last section, the
102 main findings of the study are highlighted and discussed.

103 **2. Materials**

104 *2.1. Pollen dataset*

105 Slide images were captured using a NIKON E200 microscope and a cam-
106 era NIKON DS-Fi1. Images of isolated pollen grains were obtained manually.
107 The complete procedure for image acquisition comprised several steps. Balls
108 of pollen collected by bees were dissolved with glycerogelatin drops and pre-
109 pared in slides sealed with a coverslip. A 40x magnification was used to
110 acquire images of the slide containing several pollen grains. The acquisition
111 consisted in stacks with 31 images of the slide in order to ensure an optimum
112 focus. The best focused slide was identified by an expert. Subsequently,
113 pollen grains were manually extracted from it by defining a rectangular re-
114 gion. As a result, the dimensions of the pollen images varied from one to
115 another. Pollen samples from 15 different taxa from the mediterranean area
116 were captured: 1) *Aster*, 2) *Brassica*, 3) *Campanulaceae*, 4) *Carduus*, 5) *Cas-*
117 *tanea*, 6) *Cistus*, 7) *Cytisus*, 8) *Echium*, 9) *Ericaceae*, 10) *Helianthus*, 11)
118 *Olea*, 12) *Prunus*, 13) *Quercus*, 14) *Salix* and 15) *Teucrium*. The database
119 analyzed in this study was composed of 120 brightfield microscopy images
120 per pollen taxon, resulting in a total of 1800 images. Figure 1 depicts an
121 example for each taxon included in our research.

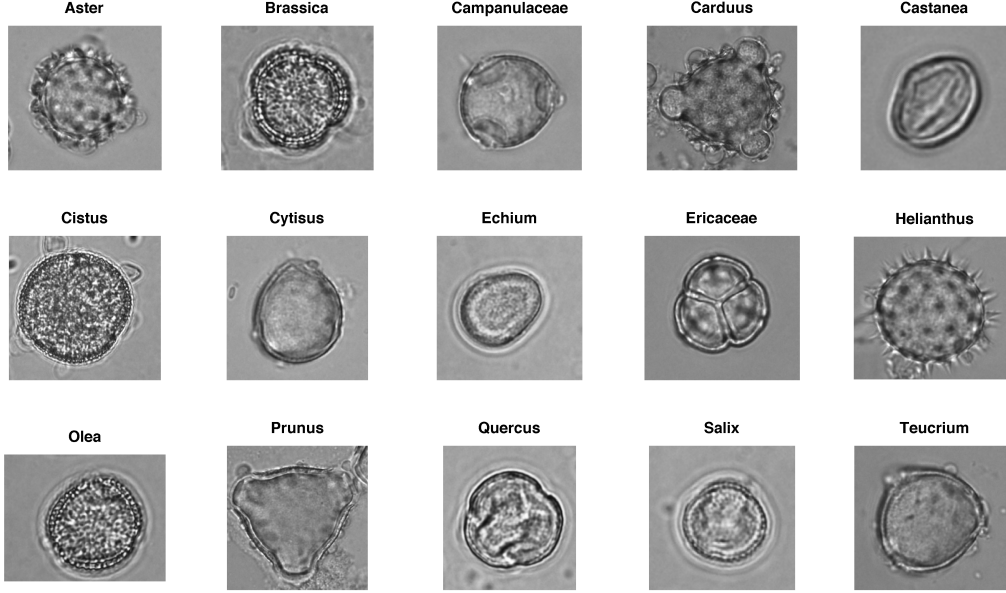


Figure 1: Sample images of the pollen taxa analyzed in our study.

122 3. Methods

123 In our study, the discriminant capability of texture in automatic pollen
 124 identification is evaluated. Hence, pollen grain images are assigned to one of
 125 several categories (taxa) according to their texture properties. We propose a
 126 pattern recognition-based methodology to perform such a classification task.
 127 It is composed of four different stages: segmentation and preprocessing, tex-
 128 ture feature extraction, dimensionality reduction and classification. Figure
 129 2 provides an schematic diagram of the proposed methodology.

130 3.1. Segmentation and preprocessing

131 The texture of a pollen grain is given by the elements characterizing its
 132 surface or exine. In order to perform texture analysis of the pollen image,
 133 segmentation was carried out to separate the target object (i.e., the grain)

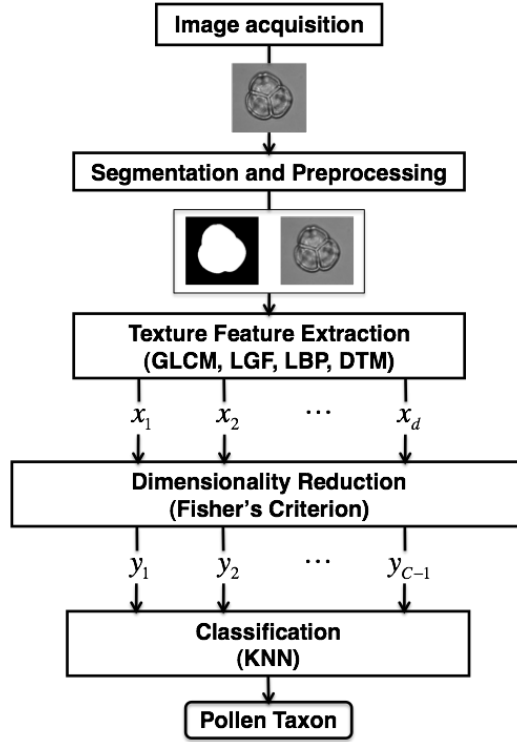


Figure 2: A scheme of the methodology adopted for automatic identification of the pollen taxon based on texture analysis. From the input image, four main processing stages are identified: segmentation and preprocessing, texture feature extraction, dimensionality reduction and classification.

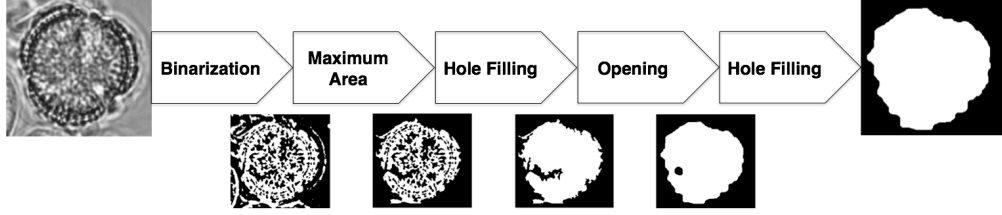


Figure 3: Segmentation process for the identification of the pollen grain in the input image.

134 from the background. A binary mask was obtained for each pollen image,
 135 indicating those pixels contained in the texture of interest. Segmentation
 136 involved several steps:

- 137 1. Binarization. The image was thresholded using an Otsu-based method
 138 (Otsu, 1975).
- 139 2. Maximum area. The region with the biggest area was retained.
- 140 3. Hole filling. Any hole in the selected region was filled.
- 141 4. Opening. The border of the region was defined using erosion and dila-
 142 tion operators.
- 143 5. Hole filling. Any remaining hole was filled.

144 Figure 3 illustrates each step of the segmentation process for one of the
 145 images in our dataset.

146 In addition to binary segmentation, preprocessing was applied before tex-
 147 ture feature extraction from the pollen image. Preprocessing is intended as
 148 a normalization stage that produces a new version of the image, so that it
 149 can be effectively processed by any of the four considered texture methods
 150 (GLCM, LBP, LGF and DTM). To this end, different requirements had to

151 be addressed in the preprocessing stage due to the distinct nature of these
 152 methods. First, unlike GLCM and LBP, which involve the evaluation of each
 153 image pixel individually, LGF and DTM perform global analysis of the im-
 154 age by simultaneously processing the entire set of pixels (Field et al., 1987;
 155 Mukundan et al., 2001). Thus, for a fair comparison (classification) of the
 156 extracted features, all the images in the database must have the same di-
 157 mension. Second, square images are recommended for an effective evaluation
 158 using LGF and DTM. Indeed, this assumption was made for the definition
 159 of the DTM-based texture descriptor employed in our study (Marcos and
 160 Cristóbal, 2013). Moreover, square images enable the use of the same number
 161 of scales for each direction in LGF banks. Third, LGF analysis is performed
 162 in the frequency domain from the spectral representation of both the image
 163 and the corresponding bank of filters. Hence, power-of-two sized images are
 164 required to optimize the performance of the method. Fourth, the size of the
 165 images must be properly limited as it influences the computational load of
 166 the evaluated texture methods. This is specially relevant for those methods
 167 based on a pixel-by-pixel analysis of the image like GLCM and LBP.

168 To fulfill these requirements, the first operation of the preprocessing stage
 169 consisted in trimming each image in the dataset, and its corresponding mask,
 170 to remove the maximum quantity of background. Subsequently, the largest
 171 image dimension (L_0) (either rows or columns) among the trimmed images
 172 was identified. It determines the minimum size of a square capable of includ-
 173 ing any of the pollen grains in the dataset. Once (L_0) has been obtained,
 174 the following steps were successively applied to each of the original pollen
 175 images:

- 176 1. Trimming. Both the image and its binary mask were trimmed.
- 177 2. Background removal. The trimmed image was multiplied by its corre-
178 sponding mask to set background pixels to 0.
- 179 3. Mean removal. The mean value of the pixels in the region of interest
180 was subtracted, resulting in a zero-mean texture.
- 181 4. Image padding. New background pixels (rows and columns) with zero
182 value were added in order to obtain an image of size $L_0 \times L_0$.
- 183 5. Resizing. The image was resized to $L \times L$ pixels, where L is a power-
184 of-two integer.

185 We found $L_0 = 450$ pixels while the final dimension L was set to 128
186 pixels to achieve an affordable computation time. The same downsampling
187 factor was applied to every image in the dataset. As a result, the prepro-
188 cessing stage preserved the aspect ratio between the sizes of different original
189 pollen grains. Finally, it is worth noting that the normalization implemented
190 by preprocessing enables each of the four texture methods to process the
191 same version of the pollen image, ensuring an unbiased comparative analysis
192 between them.

193 3.2. Feature extraction

194 The feature extraction stage aims to characterize the pollen image using a
195 set of measurements or features. This set is commonly referred to as a feature
196 pattern (Duda et al., 2012). In our study, texture features were employed to
197 define representative attributes of the pollen taxa in order to perform clas-
198 sification. The utility of the texture attributes captured by means of four
199 different methods was evaluated: GLCM, LGF, LBP and DTM.

200

201 3.2.1. Gray-level co-occurrence matrices (GLCM)

202 GLCM analysis is based on the assumption that texture information is con-
 203 tained in the spatial relationship between gray levels (Haralick et al., 1973).
 204 For the computation of GLCM, suppose that each pixel $I(x, y)$ in the image
 205 is assigned to one of B gray levels. Hence, the co-occurrence matrix P_{ij} is
 206 obtained by assessing all the possible combinations between two intensity
 207 levels $(i, j = 1, \dots, B)$. Here, P_{ij} represents the number of occurrences of
 208 two pixels with gray levels i and j separated by a distance δ in the direction
 209 determined by the angle ϕ . Note that $P_{ij} = P_{ji}$, i.e., the occurrence of (j, i)
 210 is considered as a match when computing P_{ij} and vice versa. The resulting
 211 matrix is a function of both the distance and the angular direction between
 212 pixels, so that different matrices can be obtained by varying these param-
 213 eters. Usually, the elements of the co-occurrence matrix are normalized in
 214 order to represent probabilities, providing the relative frequency of occur-
 215 rence for a pair of gray levels. The element $p(i, j)$ denotes the probability of
 216 finding the pair of levels (i, j) in the image, which is obtained as (Haralick
 217 et al., 1973):

$$p(i, j) = \frac{P_{ij}}{\sum_{i,j}^B P_{ij}} \quad (1)$$

218 These matrices of probabilities can be used to obtain statistical features
 219 that characterize the texture (Haralick et al., 1973). As suggested in (Soh
 220 and Tsatsoulis, 1999), we chose a subset of 10 features to capture texture
 221 properties. These include energy, contrast, correlation, homogeneity, en-
 222 tropy, autocorrelation, dissimilarity, cluster shade, cluster prominence, and
 223 maximum probability.

224 In our experiments, the quantization level B was set to 8 as preceding

225 studies focused on texture analysis using GLCM (Randen and Husoy, 1999).
 226 For the displacement vector, four different values of the angle ϕ were assessed
 227 ($0^\circ, 45^\circ, 90^\circ$ and 135°) (Soh and Tsatsoulis, 1999), while the distance param-
 228 eter δ was set to 1, 2 or 3. Each of these values represented a distinct scale for
 229 texture analysis. Thus, for a given value of δ , a pattern GLCM_δ composed
 230 of 40 descriptors (10 statistical features for each of the four orientations) was
 231 obtained to characterize the texture of a pollen grain. A multiscale feature
 232 pattern of dimension 120 (GLCM_{msc}) was defined by combining the descrip-
 233 tors derived from the different values of δ .

234

235 3.2.2. Log-Gabor Filters (LGF)

236 LGF were proposed by Field et al. (1987) to overcome the limitations of
 237 conventional Gabor filters. These are characterized by a maximum band-
 238 width restricted to approximately one octave and a non-zero DC component
 239 (Gao et al., 2007). In addition, it has been proved that LGF are more
 240 consistent with measurements of mammalian visual systems indicating that
 241 the cell responses are symmetric on the log frequency scale (Field et al.,
 242 1987; Kovessi, 1999). LGF are defined in the frequency domain as Gaussian
 243 functions shifted from the origin due to the singularity of the $\log(\cdot)$ func-
 244 tion. They have a null DC component and can be optimized to produce
 245 filters with minimal spatial extent in an octave scale multiresolution scheme.
 246 Mathematically, LGF can be divided into two components referred to as
 247 radial and angular filters (Gao et al., 2007):

$$\hat{G}(\rho, \theta) = \hat{G}_\rho \hat{G}_\theta = e^{-\frac{1}{2} \left[\frac{\log\left(\frac{\rho}{u_0}\right)}{\log\left(\frac{\alpha\rho}{u_0}\right)} \right]^2} e^{-\frac{1}{2} \left[\frac{(\theta - \theta_0)}{\alpha_\theta} \right]^2} \quad (2)$$

248 where ρ and θ represent the polar coordinates, u_0 is the central frequency,
 249 θ_0 is the orientation angle, and the parameters α_ρ and α_θ determine the
 250 scale and the angular bandwidth, respectively. In our experiments, we set
 251 $\alpha_\rho = 0.75$ and $\alpha_{theta} = \frac{\pi}{6}$ since previous studies showed that these values
 252 result in minimal overlap among scales one octave apart (Nava et al., 2012).
 253 These values resulted in a bank of 24 filters distributed in 4 scales and 6
 254 orientations. In order to better cover the Fourier plane, even scales were
 255 rotated by a constant factor consisting of the half a distance between filter
 256 centers (Gross and Koch, 1995).

257 To characterize the response of the image to the LGF bank, the first four
 258 standard moments of the filtered image at each scale and orientation were
 259 computed (Schwartz et al., 2012). These moments include mean, variance,
 260 skewness and kurtosis. They quantify the central tendency, the degree of
 261 dispersion, the asymmetry and the peakedness of the distribution of samples
 262 in the filtered image. As a result, a feature vector composed of 96 elements
 263 (4 features for each of the 24 filters) was used to describe the texture of the
 264 pollen grain.

265

266 3.2.3. Local binary patterns (LBP)

267 The LBP operator assumes that texture is composed of different patterns or
 268 local spatial structures that are repeated in the image (Ojala et al., 2002).
 269 The LBP detects them by acting as a template mask that is evaluated at each
 270 image pixel. An estimate of the probability of occurrence of these structures
 271 is obtained by means of a histogram, which is used to describe the texture
 272 (Ojala et al., 2002).

273 To compute the LBP of an image, a neighbourhood of P pixels equally
 274 distributed on a circumference of radius R around a central pixel (g_c) is
 275 considered. The value of g_c is taken as a threshold to obtain the LBP, which
 276 is expressed as:

$$LBP_{P,R}(g_c) = \sum_{p=0}^{P-1} H(g_p - g_c) 2^p \quad (3)$$

277 where g_p ($p = 0, \dots, P-1$) are the values of the neighbours and $H(\cdot)$ is the
 278 Heaviside function. The formulation of $LBP_{P,R}$ yields a total of 2^P different
 279 patterns. The probability associated with each of them is estimated by means
 280 of its relative frequency, leading to a histogram with 2^P bins. However,
 281 for P high enough, a large number of bins is obtained, resulting in sparse
 282 histograms.

283 It was observed that certain patterns, called as uniform patterns, present
 284 improved capabilities for texture discrimination (Ojala et al., 2002). Uniform
 285 patterns are characterized for a maximum of two bit transitions in their
 286 binary representation as measured by the operator $U(LBP_{P,R})$, which is
 287 given by:

$$U(LBP_{P,R}(g_c)) = |H(g_{P-1} - g_c) - H(g_0 - g_c)| + \sum_{p=1}^{P-1} |H(g_p - g_c) - H(g_{p-1} - g_c)| \quad (4)$$

288 The uniform LBP ($LBP_{P,R}^{uni}$) is then defined as (Ojala et al., 2002):

$$LBP_{P,R}^{uni}(g_c) = \begin{cases} \sum_{p=0}^{P-1} H(g_p - g_c) & \text{if } U(LBP_{P,R}(g_c)) \leq 2 \\ P + 1 & \text{otherwise} \end{cases} \quad (5)$$

289 As a result, $LBP_{P,R}^{uni}$ produces a $(P+2)$ -bin histogram that characterizes
 290 the pollen texture. In our study, three different configurations, $P = 8 - R =$

1, $P = 16 - R = 2$ and $P = 24 - R = 3$, were adopted to evaluate texture in several scales (Ojala et al., 2002). A multiscale texture descriptor (LBP_{msc}) was obtained by combining the feature patterns from these three different scales.

3.2.4. Discrete Tchebichef moments (DTM)

The set of DTM provides a unique representation of an image $I(x, y)$ in the space spanned by Tchebichef kernels. The moment $T_{pq}(p, q = 0, 1, \dots, L - 1)$ of order $s = p + q$ is defined as (Mukundan et al., 2001):

$$T_{pq} = \frac{1}{\tilde{\eta}(p)\tilde{\eta}(q)} \sum_{x=0}^{L-1} \sum_{y=0}^{L-1} \tilde{t}_p(x) \tilde{t}_q(y) I(x, y), \quad (6)$$

where $\tilde{t}_n(x)$ is the scaled Tchebichef polynomial of degree n and $\tilde{\eta}(n)$ is its squared norm (Mukundan, 2004). The function $r_{pq}(x, y) = \tilde{t}_p(x) \tilde{t}_q(y)$ denotes the two-dimensional Tchebichef kernel.

Recently, a texture descriptor has been proposed based on the properties of discrete Tchebichef kernels (Marcos and Cristóbal, 2013). From equation 6, the magnitude of T_{pq} quantifies the correlation between the original image $I(x, y)$ and the kernel $r_{pq}(x, y)$, which has an oscillating profile. Thus, this magnitude will be higher for images characterized by repetitive patterns occurring at a similar rate to $r_{pq}(x, y)$. This is a relevant property for texture analysis since texture involves the spatial repetition of intensity patterns (Tuceryan and Jain, 1993). As kernels of the same order are characterized by similar frequency content, i.e., similar varying patterns along different directions (Teh and Chin, 1988), a description of the texture attributes is obtained by assessing the dependence of the total moment magnitude on the

314 kernel order s (Marcos and Cristóbal, 2013):

$$M(s) = \sum_{p+q=s} |T_{pq}|, \quad (s = 0, 1, \dots, 2L - 2). \quad (7)$$

315 The value of $M(s)$ evaluates the similarity between the original image
316 and the varying patterns implemented by s -order Tchebichef kernels. The
317 analysis based on DTM yields a vector $M(s)$ of length $2L - 1$ that describes
318 the texture of the pollen grain.

319 3.3. Dimensionality reduction

320 Feature extraction enables to summarize the information in the pollen
321 texture by means of a vector (pattern) of features. This vector could be di-
322 rectly used for pollen classification. However, dimensionality reduction was
323 performed through a twofold purpose. First, it represents a normalization
324 stage for the obtained feature vector since its dimension depends on the
325 method adopted for texture analysis. Dimensionality reduction avoids this
326 dependence by providing a new vector whose dimension can be controlled by
327 the user. As the new dimension is smaller than that of the original feature
328 vector, a more compact representation of the pollen texture is obtained. Sec-
329 ond, reducing the dimension of the input feature space prevents overfitting.
330 As detailed in (Bishop, 1995), in order to obtain an accurate statistical de-
331 scription of the problem, the size of the training set should be exponentially
332 increased with the dimensionality of the input space. Commonly, a limited
333 dataset is available in real applications such as the proposed pollen classifi-
334 cation problem. Thus, reduced dimensionality may be advantageous for the
335 posterior classification stage.

336 We used the conventional Fisher’s discriminant analysis (FDA) to perform
337 dimensionality reduction (Fisher, 1936; Bishop, 1995). For a multiclass
338 classification problem with C possible categories, the original feature vector
339 $\mathbf{x} = (x_1, x_2, \dots, x_d)$ of dimension d ($d \geq C$) is mapped onto a new space of
340 dimension $d' = C - 1$. The transformation matrix \mathbf{W} is obtained according to
341 the Fisher’s criterion, which maximizes the ratio of the interclass variability
342 to the intraclass variability for the transformed samples. Mathematically, it
343 is expressed as follows (Bishop, 1995):

$$J(\mathbf{W}) = \frac{|\mathbf{W}^T \mathbf{S}_B \mathbf{W}|}{|\mathbf{W}^T \mathbf{S}_W \mathbf{W}|} \quad (8)$$

344 where \mathbf{S}_B and \mathbf{S}_W are the between-class and the within-class scatter matrices,
345 respectively. The columns of \mathbf{W} are given by the eigenvectors associated with
346 the d' largest eigenvalues of the matrix $\mathbf{S}_W^{-1} \mathbf{S}_B$. Therefore, the projection \mathbf{y}
347 of the original feature vector \mathbf{x} is obtained as (Bishop, 1995):

$$\mathbf{y} = \mathbf{W}^T \mathbf{x}. \quad (9)$$

348 3.4. Classification

349 In the final stage, the vector \mathbf{y} resulting from dimensionality reduction is
350 labelled as one of the 15 pollen classes considered in our study. According to
351 the Bayes’ decision rule, it must be assigned to the class ω_i for which the pos-
352 terior probability $p(\omega_i | \mathbf{y})$ ($i = 1, \dots, C$) is the highest in order to minimize
353 the risk of misclassification. Multivariate analysis was performed to define
354 decision boundaries in the d' -dimensional ($d' = C - 1$) input space using the
355 k -nearest neighbour (KNN) approach. KNN is based on the approximation

356 of the probability density function of a variable from a finite set of samples
 357 as described in the following expression (Bishop, 1995):

$$p(\mathbf{y}) \approx \frac{K}{NV}. \quad (10)$$

358 where K represents the total number of samples that are found in a volume
 359 V centred on \mathbf{y} . In a classification problem, this procedure can be used to
 360 model the class-conditional density $p(\mathbf{y}|\omega_i)$ of each category ω_i as:

$$p(\mathbf{y}|\omega_i) \approx \frac{K_i}{N_i V}, \quad (11)$$

361 where K_i represents the total number of samples belonging to class ω_i that
 362 are found in a volume V centred on \mathbf{y} , given that N_i is the total number of
 363 training samples of class ω_i .

364 Since the prior probability of a class is estimated as the proportion of
 365 samples belonging to that class, i.e., $p(\omega_i) \approx \frac{N_i}{N}$, the posterior probability
 366 $p(\omega_i|\mathbf{y})$ can be obtained from the Bayes' theorem as follows (Bishop, 1995):

$$p(\omega_i|\mathbf{y}) = \frac{p(\mathbf{y}|\omega_i)p(\omega_i)}{p(\mathbf{y})} \approx \frac{K_i}{K} \quad (12)$$

367 Once posterior probabilities are known, classification is carried out by
 368 applying the maximum a posteriori probability rule described before. For
 369 this purpose, the K nearest neighbours to the input vector are retained and
 370 the class that includes the highest number of neighbours is selected. As a
 371 result, the KNN classifier defines a non-linear decision boundary in the input
 372 space.

373 The smoothing parameter K influences the complexity of the decision
 374 boundary defined by the classifier, with high bias (smooth boundaries) cor-

375 responding to high values of K (Bishop, 1995). Hence, increasing K is
 376 required to ensure a reliable estimation of the posterior probabilities. On the
 377 other hand, K must be sufficiently small to be sure that $p(\mathbf{y}|\omega_i)$ is similar at
 378 all the neighbours of the point \mathbf{y} . This trade-off leads to a compromise value
 379 of K given by a small fraction of the available samples (Duda et al., 2012).
 380 According to this analysis, K was set to 20 in our study, which approximately
 381 represents 1% of the images in our dataset.

382 **4. Results**

383 *4.1. Experiments on the pollen database*

384 In our experiments, accuracy was adopted as the performance measure to
 385 evaluate the discriminant capability of a feature pattern in the pollen classi-
 386 fication problem. Accuracy provides the probability of correct classification
 387 for a given pollen image. This probability is approximated by the percentage
 388 of pollen grains correctly classified (Bishop, 1995). In the present study,
 389 ten-fold cross-validation was applied to estimate classification accuracy from
 390 the original dataset of 1800 images (Bishop, 1995; Duda et al., 2012).

391 In our experiments, several classification algorithms were implemented ac-
 392 cording to the methodology described before. Hence, in order to determine
 393 the most advantageous configuration of GLCM and LBP, different scales of
 394 analysis were evaluated for these methods. Additionally, we assessed the util-
 395 ity of the combination of the four texture methods in the pollen identification
 396 problem. For this purpose, among all the evaluated configurations of GLCM
 397 and LBP, those with the highest performance were selected to be combined
 398 with LGF and DTM features, resulting in the definition of a new texture

399 feature pattern (ALL). Finally, to measure the impact of Fisher dimension-
400 ality reduction on the system performance, two classification schemes were
401 compared: one using Fisher dimensionality reduction followed by the KNN
402 algorithm (FDA-KNN), and another one using a simple KNN classifier on
403 the raw texture features (i.e., without any kind of dimensionality reduction).

404 In the following Table 1, the results achieved by the classification algo-
405 rithms evaluated in our experiments are summarized. Note that no result
406 is reported for $\text{LBP}_{8,1}$ with FDA-KNN classification as the dimensionality
407 reduction stage is not applicable to feature spaces of dimension lower than
408 $C = 15$. Several observations can be made from the obtained results. First,
409 configurations including Fisher dimensionality reduction outperformed those
410 based on a simple KNN classifier fed with raw texture features. This reveals
411 that the dimensionality reduction stage enabled higher generalization capa-
412 bility (reduced overfitting) by enforcing smooth decision boundaries between
413 pollen categories in the input feature space. Second, the comparative analy-
414 sis between the four texture methods shows that LGF and DTM yielded the
415 highest classification performance. They achieved a correct classification rate
416 above 92% in the pollen identification problem. GLCM_1 and LBP_{msc} were
417 the configurations with the highest performance for GLCM and LBP meth-
418 ods, providing an acceptable accuracy of 89.94% and 89.83%, respectively.
419 Third, useful non-redundant texture features can be derived from GLCM
420 and LBP analysis at different scales. Regarding the LBP method, the mul-
421 tiscala approach (LBP_{msc}) outperformed single scale configurations for both
422 FDA-KNN and KNN classification schemes. Therefore, the combination of
423 texture features captured by LBP at different scales provides a richer de-

424 description of the texture attributes when compared to single scale analysis.
 425 In the case of GLCM, multiscale analysis led to improved classification per-
 426 formance when no dimensionality reduction was applied. An accuracy of
 427 65.06% was achieved by GLCM_{msc} while single scale configurations provided
 428 up to 60.39% accuracy. This reflects that complementary information is de-
 429 rived from GLCM analysis at distinct scales. However, no improvement was
 430 observed in the multiscale approach of GLCM when FDA-KNN classification
 431 was used. This result may be motivated by the increased difficulty of the
 432 dimensionality reduction task. In the multiscale approach, the dimension of
 433 the original feature space (120 features) is considerable higher than in single
 434 scale configurations (40 features), resulting in a higher degree of overlap-
 435 ping between categories in the transformed space (Bishop, 1995). Fourth,
 436 the combination approach (ALL) yielded 94.83% accuracy, improving the
 437 performance individually achieved by each texture method. The ALL pat-
 438 tern was obtained as the concatenation of GLCM_1 , LGF, LBP_{msc} and DTM
 439 patterns. Observe that, according to our results, GLCM_1 and LBP_{msc} were
 440 selected as the optimum configurations of GLCM and LBP, respectively. The
 441 result achieved by the ALL approach could be initially expected as a more
 442 robust and detailed description of the pollen texture is obtained by com-
 443 bining information from different methods. Specifically, we found that the
 444 ALL pattern led to 93 misclassified pollen images out of 1800 samples, what
 445 represents a high percentage of correct classification.

446 Table 2 details the results provided by the selected configurations of the
 447 four texture methods (GLCM_1 , LGF, LBP_{msc} and DTM) and their com-
 448 bination into the ALL feature pattern. For a thorough analysis of their

	FDA-KNN	KNN
LBP_{8,1}	-	81.83
LBP_{16,2}	84.89	80.28
LBP_{24,3}	87.83	80.17
LBP_{msc}	89.83	84.06
GLCM₁	89.94	57.06
GLCM₂	84.39	57.11
GLCM₃	85.83	60.39
GLCM_{msc}	85.11	65.06
LGF	92.50	90.06
DTM	92.06	89.83
ALL	94.83	83.78

Table 1: Total classification accuracy achieved by the evaluated texture feature patterns in the pollen identification problem.

449 performance, the classification accuracy achieved on each pollen category is
450 reported.

451 The results reflect that most of the errors corresponded to ‘*Cytisus*’ (7)
452 samples, for which all the methods achieved lower accuracy rates. Other cat-
453 egories that also presented marked difficulties in classification were ‘*Quercus*’
454 (13), for which LGF provided reduced accuracy, and ‘*Teucrium*’ (15), which
455 was specially difficult for GLCM₁ and LBP_{msc}. Conversely, the highest cor-
456 rect classification rate was achieved on ‘*Castanea*’ (5), ‘*Cistus*’ (6), ‘*Echium*’
457 (8) and ‘*Salix*’ (14) categories. The combination of the four texture descrip-
458 tors resulted in a substantial decrease in the number of errors. For most of
459 the categories, a positive increment in classification accuracy was observed
460 with respect to any of the methods. It is worth noting that the increment
461 was specially large in ‘*Cytisus*’ (7), ‘*Quercus*’ (13) and ‘*Aster*’ (1), for which
462 an improvement higher than 5 percentage points was obtained. As observed
463 in the individual assessment of the methods, the lowest accuracy values were
464 still achieved on ‘*Cytisus*’ (7) and ‘*Teucrium*’ (15) samples.

465 4.2. Analysis of the results

466 To identify error flows between categories, Figure 4 depicts the error
467 matrices for the four evaluated texture methods. They provide a graphical
468 representation of the confusion matrices once the main diagonal has been re-
469 moved. As a result, only the number of misclassified samples is observed. The
470 error matrices show that ‘*Cytisus*’ (7) samples, for which all the evaluated
471 algorithms provided reduced classification accuracy, tended to be labeled as
472 ‘*Campanulaceae*’ (3) or ‘*Teucrium*’ (15). The similarity between ‘*Cytisus*’
473 (7) and ‘*Teucrium*’ (15) pollen samples also led to a significant number of

		Texture Features				
		GLCM ₁	LGF	LBP _{msc}	DTM	ALL
Taxon	<i>Aster</i>	89.17	95.00	94.17	91.67	100.00
	<i>Brassica</i>	95.83	89.17	82.50	94.17	90.00
	<i>Campanulaceae</i>	85.83	93.33	86.67	92.50	90.83
	<i>Carduus</i>	98.33	97.50	89.17	95.83	99.17
	<i>Castanea</i>	99.17	100.00	100.00	99.17	100.00
	<i>Cistus</i>	99.17	99.17	97.50	99.17	99.17
	<i>Cytisus</i>	73.33	77.50	69.17	71.67	85.83
	<i>Echium</i>	94.17	98.33	98.33	99.17	98.33
	<i>Ericaceae</i>	95.00	89.17	93.33	88.33	99.17
	<i>Helianthus</i>	81.67	93.33	90.83	94.17	90.83
	<i>Olea</i>	93.33	91.67	91.67	92.50	96.67
	<i>Prunus</i>	98.33	99.17	92.50	93.33	96.67
	<i>Quercus</i>	88.33	81.67	90.83	87.50	95.00
	<i>Salix</i>	95.00	95.83	95.83	96.67	99.17
	<i>Teucrium</i>	62.50	85.83	75.00	85.00	81.67
	Total	89.94	92.50	89.83	92.06	94.83

Table 2: Accuracy on each pollen category and total accuracy for the most relevant classification algorithms.

classification errors in the opposite direction. Hence, a common trend was observed in the four evaluated methods since misclassified ‘*Teucrium*’ (15) images were usually labelled as ‘*Cytisus*’ (7) by all of them. In addition to the common difficulties found with ‘*Cytisus*’ (7) samples, other particular results were observed. For instance, LGF and LBP labelled as ‘*Olea*’ (11) a relevant number of ‘*Quercus*’ (13) and ‘*Brassica*’ (2) samples, respectively. In addition, GLCM and LBP exhibited particular difficulties between ‘*Aster*’ (1) and ‘*Helianthus*’ (10).

The detected error flows reflect a coherent behaviour of our classification algorithms. As shown in Figure 1, these flows connect pollen taxa with a similar appearance. As an example, consider the pairs given by ‘*Cytisus*’ (7) and ‘*Teucrium*’ (15), ‘*Aster*’ (1) and ‘*Helianthus*’ (10), or ‘*Brassica*’ (2) and ‘*Olea*’ (11). At this point, it is interesting to analyze the effect of the combination approach on the observed error flows. Table 3 details the confusion matrix obtained when a single feature pattern with all the texture features is used to classify pollen samples. As can be appreciated, most of the errors associated with each individual texture method are removed by adopting the combination approach. Indeed, only the error flow between ‘*Cytisus*’ (7) and ‘*Teucrium*’ (15) seems to be substantial. Therefore, the combination of different texture features provided the most efficient characterization of the pollen taxon.

According to our results, LGF and DTM capture more useful information about the pollen taxon, resulting in higher classification accuracy than the other texture descriptors. These two methods are based on the analysis of the image using a bank of filters placed at different frequency bands (Field

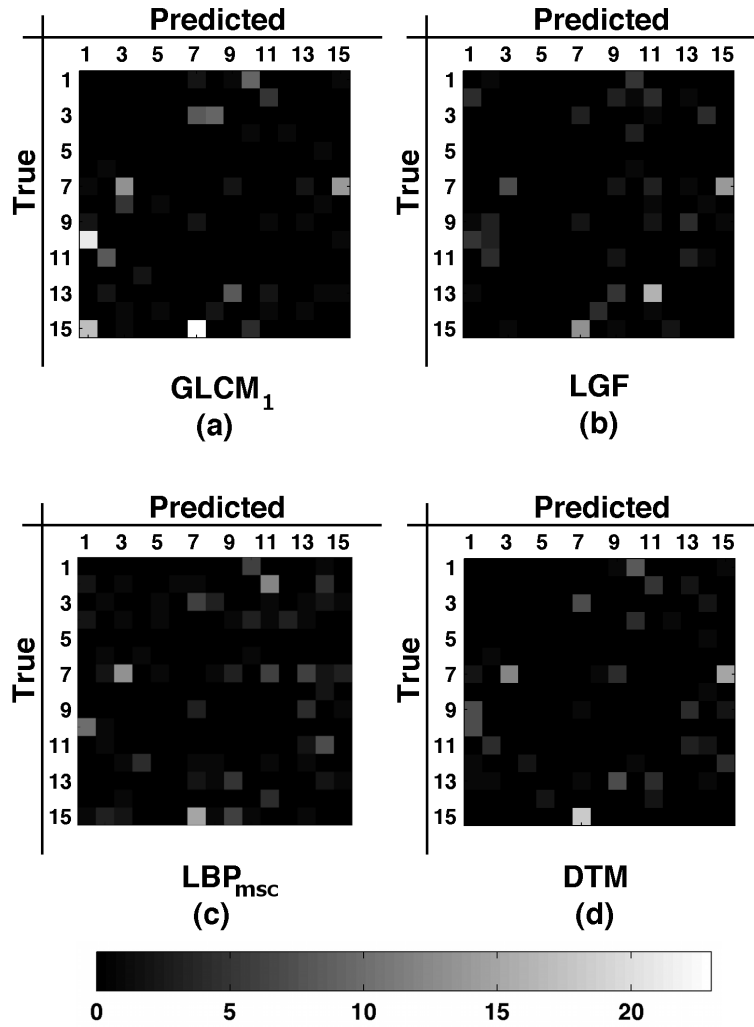


Figure 4: Error matrices for each texture method.

		Predicted														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
True	1	120														
	2		108									12				
	3			109				8							3	
	4				119								1			
	5					120										
	6						119					1				
	7			7				103		1					1	8
	8								118						2	
	9							1		119						
	10	10									109		1			
	11		3									116			1	
	12	1			1						2		116			
	13		1					1		2				114	2	
	14					1									119	
	15			1				20		1						98

Table 3: Confusion matrix resulted from the combination of the four texture methods.

499 et al., 1987; Marcos and Cristóbal, 2013). This suggests that the spectral
 500 content of a pollen image will vary from a category to another while it will
 501 be similar for samples in the same category. To analyze this behaviour, we
 502 computed the average power spectral density (PSD) of pollen images in each
 503 category, which is depicted in Figure 5. The spectra exhibit approximate
 504 radial symmetry, indicating that the pollen textures are not associated with a
 505 specific orientation. In addition, the resulting figure shows that the energy of
 506 a pollen image is composed of different frequency components depending on
 507 its taxon. For instance, high frequency components are observed in ‘*Cistus*’
 508 (6) pollen images while ‘*Aster*’ (1) and ‘*Campanulaceae*’ (3) images do not
 509 have a significant energy content at high frequencies. On the other hand,
 510 ‘*Castanea*’ (5) images tend to have lower energy than pollen samples from
 511 other taxa as reflected by the small magnitude of the averaged spectrum. To
 512 appreciate these differences, Figure 5 shows the profile of the PSD along the
 513 x -axis (i.e., the frequency coordinate f_y is set to 0). It is worth noting that the
 514 choice of the orientation does not have a significant influence on this analysis
 515 as the spectra do not reveal substantial variations along the angle coordinate.
 516 Categories ‘*Campanulaceae*’ (3), ‘*Cytisus*’ (7) and ‘*Teucrium*’ (15), among
 517 which the evaluated methods performed most of the classification errors,
 518 have very similar PSD profiles. On the contrary, other categories such as
 519 ‘*Castanea*’ (5), ‘*Cistus*’ (6) or ‘*Echium*’ (8) exhibit a profile significantly
 520 different to the rest. As a result, they can be more easily identified as reflected
 521 by the classification accuracy close to 100% achieved by LGF and DTM on
 522 these categories.

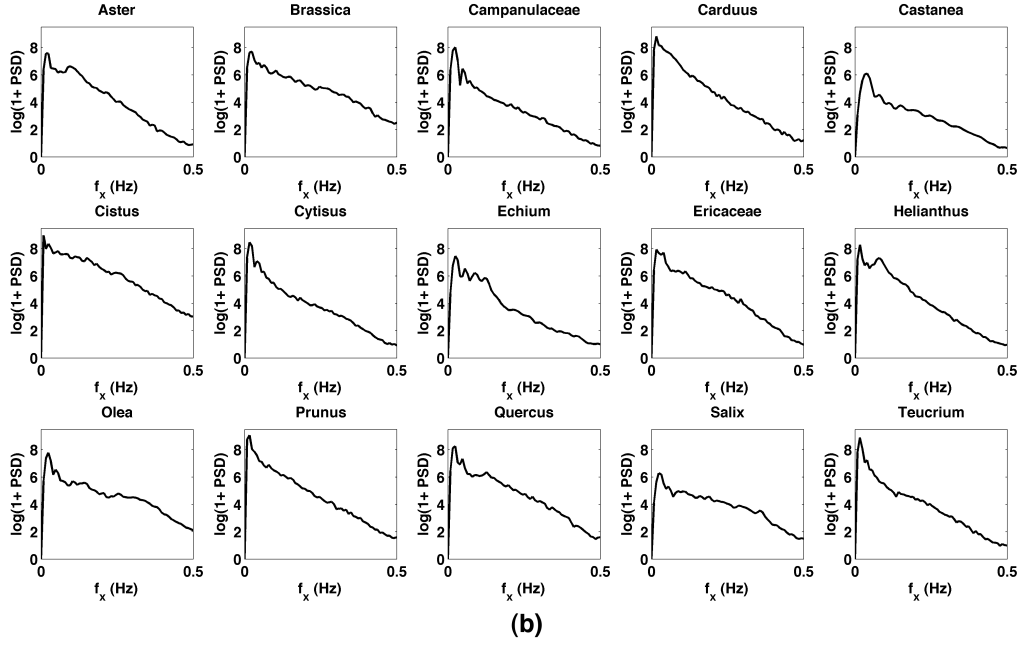
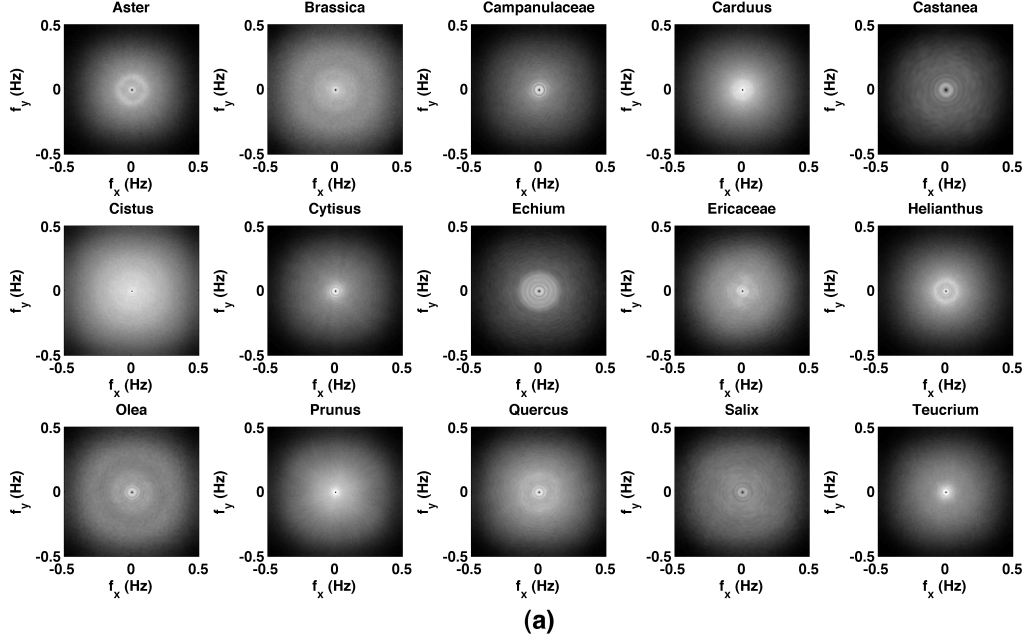


Figure 5: Spectral content of pollen images in each category: (a) averaged PSD of images in each category and (b) profile of the averaged PSD along the f_x coordinate.

523 5. Discussion and conclusions

524 A detailed analysis on the role of texture in pollen taxon characterization
525 was presented. In addition to conventional texture feature extraction based
526 on GLCM, other methods including LGF, LBP and DTM were assessed in
527 our study. Furthermore, the complementarity between these texture features
528 was explored by means of their combination. A methodology involving di-
529 mensionality reduction based on FDA and KNN classification was proposed
530 to identify pollen samples from 15 different taxa. The obtained results re-
531 flect the superior performance of LGF and DTM texture descriptors as well as
532 the utility of the combination approach in the proposed pollen classification
533 problem.

534 Our experiments support the conclusion that texture is a distinctive char-
535 acteristic of the pollen taxon. Note that 95% of the grains in a dataset com-
536 posed of 1800 samples from 15 different taxa were correctly identified by only
537 using texture features. In particular, it should be appreciated that texture
538 analysis enabled high discrimination between pollen samples from taxa with
539 an increased degree of similarity. For instance, consider the pairs given by
540 ‘*Brassica*’ (2) and ‘*Olea*’ (11) as well as ‘*Aster*’ (1) and ‘*Helianthus*’ (10)
541 (see Figure 1). In the case of ‘*Cytisus*’ (7) and ‘*Teucrium*’ (15), which were
542 responsible for most of the errors of our algorithms, a significant classifi-
543 cation accuracy over 80% has been achieved. On the other hand, we have
544 demonstrated that pollen textures from distinct categories tend to exhibit
545 differences in their spectral content. Hence, texture descriptors based on
546 spectral analysis such as LGF and DTM have shown to be capable of captur-
547 ing these differences, reaching significantly high accuracy in the pollen clas-

sification problem. However, we found that the most effective representation of the pollen taxa was not given by a single texture descriptor. Instead, the combination of different texture features resulted in improved classification accuracy. This represents a more complex approach since several processing techniques must be applied to perform texture feature extraction from the original pollen image. Nevertheless, our results show that uncorrelated texture features complement themselves in order to enable the pollen identification task.

Previously, other researchers highlighted the relevance of texture in pollen identification. Commonly, GLCM analysis was proposed to extract texture attributes (Langford et al., 1990; Li and Flenley, 1999; Kaya et al., 2013; Rodriguez-Damian et al., 2006; Zhang et al., 2004, 2005). These features were applied individually or in combination with other descriptors for automatic pollen classification. The achieved accuracies ranged from 88% (10 categories) (Kaya et al., 2013) to 100% (4 categories) (Li and Flenley, 1999). A fair comparison of our results with those reported in preceding studies cannot be carried out since different databases were analyzed. However, two main observations can be made. First, a comparable or higher number of samples and categories was included in our research. This contributes to increase the reliability of our experimental results and reflects the validity of the proposed texture methods in the analysis of microscopy pollen images. Second, although most of the previous studies used GLCM for texture characterization, other texture descriptors such as LGF or DTM based on the spectral properties of the pollen image have proven to be more efficient for capturing distinctive information about the taxa.

573 The main handicap for the development of an automatic pollen identifi-
574 cation method is the huge number of distinct plant species and pollen taxa.
575 As a result, the implementation of a universal method for pollen recognition
576 seems an intractable task. Instead, a subset of pollen taxa including those
577 involved in a specific application or context is usually considered. In our
578 study, 15 different taxa corresponding to honey-bee pollen from the mediter-
579 ranean area were analyzed. However, it must be noted that the evaluated
580 texture methods can be applied to other domains in which automatic pollen
581 identification is required. Indeed, texture features provide several advantages
582 when compared with other techniques for this purpose. For instance, unlike
583 shape or morphological features, texture analysis adapts to the evaluation
584 of fossil pollen samples that may be broken (Li et al., 2004). In addition,
585 texture avoids the dependence on the position of the pollen grain, which is
586 crucial for methods based on the detection of pores. Furthermore, no com-
587 plex equipment is required to acquire pollen images such as those evaluated
588 in our study, which were captured through brightfield microscopy. Hence,
589 the proposed methods could be implemented in a specific software module
590 coupled to the microscope, enabling the analysis of a large number of pollen
591 samples in a reasonable time. This would overcome the limitation of spec-
592 troscopy techniques (Pappas et al., 2003; Ivleva et al., 2005; DellAnna et al.,
593 2009; Schulte et al., 2008), which require more complex optical elements
594 (spectrometers) and preparation to analyze the composition of the pollen
595 samples.

596 Despite the advantages from texture analysis, several limitations can be
597 found in our study. Although the available image dataset includes a relevant

number of taxa and samples, its size seems to be insufficient for certain tasks
 involving the implementation of the classification algorithm. For instance,
 we adopted KNN with $K = 20$ for multivariate pattern classification. A
 search for the optimum K was not carried out as an independent dataset
 would be needed to avoid biased test results. In addition, previous studies
 in the field reported significant performance by means of neural networks or
 support vector machines (Li and Flenley, 1999; Rodriguez-Damian et al.,
 2006). Nevertheless, these algorithms have a considerable amount of design
 parameters and adaptable weights. A larger number of samples per category
 than that available in our research would be required to adjust them and
 prevent overfitting (Bishop, 1995). The latter must be taken into account
 in the design of classification algorithms. In our study, the strategy adopted
 to avoid overfitting was the use of dimensionality reduction based on FDA.
 Our experiments revealed that dimensionality reduction played an essential
 role to obtain classification algorithms with a high generalization capability.
 As an alternative, other procedures could have been considered in order to
 reduce the number of input features to the classifier. For instance, a smaller
 number of statistics could be derived from each of the filters employed in
 LGF or GLCM features could be averaged along the four angle directions.
 Another limitation of our research is given by the acquisition of the data, as
 images of isolated pollen grains analyzed in our study were manually obtained
 from the image of the whole slide. Further research is required in order to
 achieve a fully automatic system for pollen identification by implementing
 a software module enabling the localization of pollen grains in the original
 slide. Finally, it is worth noting that a single image was analyzed from the

623 whole stack captured for each pollen grain. Future studies could evaluate the
624 use of fusion techniques including several images at different distances from
625 the object.

626 In summary, our study reflects that the texture of the pollen exine is a dis-
627 tinguishing property of its taxon. According to our results, other methods are
628 more useful for the characterization of the pollen texture than conventional
629 first-order and secon-order statistics such as Haralick’s GLCM. Specifically,
630 we have shown that the spectral representation of pollen images enables the
631 extraction of texture features that vary from a taxon to another. Thus, tex-
632 ture descriptors based on spectral filtering such as LGF or DTM suitable
633 adapt to the pollen identification problem. Furthermore, our experiments
634 reveal that the complementarity between different texture features can be
635 exploited in order to achieve higher classification performance. Therefore,
636 we propose an exhaustive analysis of texture in image-based applications
637 pursuing automatic identification of pollen taxa.

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758 **Figure captions**

759 Figure 1. Sample images of the pollen taxa analyzed in our study.

760 Figure 2. A scheme of the methodology adopted for automatic identifi-
761 cation of the pollen taxon based on texture analysis. From the input image,
762 four main processing stages are identified: segmentation and preprocessing,
763 texture feature extraction, dimensionality reduction and classification.

764 Figure 3. Segmentation process for the identification of the pollen grain
765 in the input image.

766 Figure 4. Error matrices for the four evaluated texture methods.

767 Figure 5. Spectral content of pollen images in each category: (a) averaged
768 PSD of images in each category and (b) profile of the averaged PSD along
769 the f_x coordinate.

770 **Table captions**

771 Table 1. Total classification accuracy achieved by the evaluated texture
772 feature patterns in the pollen identification problem.

773 Table 2. Accuracy on each pollen category and total accuracy for the
774 most relevant classification algorithms.

775 Table 3. Confusion matrix resulted from the combination of the four
776 texture methods.