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1 Identifying Plant Species Using Architectural
2 Features of Leaf Microscopy Images

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Abstract

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This work proposes an analytical method to identify plant species based on microscopy images of the midrib cross-section of leaves. Unlike previous shape-based approaches based on the individual shape of external contours and cells, an architectural analysis is proposed, where the midrib is semi-automatically segmented and partitioned into histologically relevant structures composed of layers of cells and vascular structures. Using a sequence of morphological operations, a set of geometrical measures from the cells in each layer is extracted to produce a vector of features for species categorization. The method applied to a database containing 10 species of plants from the Brazilian *flora* achieved a success rate of 91.7%, outperforming other classical shape-based approaches published in the literature.

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Keywords— Automatic Species Identification, Image Analysis, Morphological

36

Features

1 Introduction

The categorization of plants into species is an important taxonomical task; it provides meaningful information about particular characteristics of a plant, which can be used in the context of biodiversity and environmental relations with other species.

Plant identification is an old problem and, classically it has been based on conventional visual characterisation of plant morphology. It is only recently that computer-based analyses have been used for this purpose (Rossatto et al., 2011; Sá Junior et al., 2011; Florindo et al., 2014; Silva et al., 2014). Computational methods are particularly interesting because they enable a more precise identification of plant leaves, especially when leaves from different species are too similar to each other and the conventional morphological features are not sufficient for their discrimination (Bruno et al., 2008; Backes et al., 2009; da Silva et al., 2015; Florindo et al., 2014). This is especially important in situations where, for example, reproductive structures are not easily obtainable like plants in vegetative phase.

Among the computational methods proposed, those using information from the leaves have become very popular, mostly because leaves can be easily found at any time. Image analysis has proven to be a powerful tool in the description of these structures (Florindo et al., 2014). Leaves are diverse and complex structures in terms of anatomy and morphology (Evert, 2006) and modern computational approaches can extract large amount of useful information from them.

Most analyses of leaf images use macroscopic features such as leaf contour and venation distribution, among others (Backes et al., 2009; Bruno et al., 2008). More recently, the analysis of microscopic features has been a subject of investigation, using images from midrib cross-sections (Silva et al., 2014). Those studies, however, used approaches to characterise leaf structure externally or internally, as indivisible elements without taking into consideration the relations between their parts (for instance, cells and vascular spaces observed in cross-sections).

Here we propose a taxonomic analysis of plant leaves, based on quantifying the architecture of the midrib as observed in microscopical cross-sections. More specifically, the midrib is characterised using adjacent layers of cells and vascular spaces. Visually, it can be noticed that cells and vascular spaces (represented in analytical terms by pixel regions called 'v-cells' or 'virtual cells') are arranged in a seemingly hierarchical order or layers such that they appear to exhibit characteristic morphological features at various depths from the leave surface. The analysis, therefore, is aimed at computing various morphological descriptors of this structural organisation (e.g. v-cell area, perimeter, Feret diameter and others) forming those layers. Those layer descriptors are averaged and the resulting set of features submitted first to a Karhunen-Loève transform to reduce the data dimensionality and then to a learning machine algorithm to classify the cases into different plant species.

When this novel approach was applied to a database of plants from the

82 Brazilian *flora*, 91.7% of the samples were successfully identified, suggesting
 83 that the proposed method might be useful for taxonomic identification. The
 84 results also suggest that the organization of v-cells within a leaf might provide
 85 useful descriptors for defining architectural metrics to investigate and compare
 86 how species might be morphologically related.

87 2 Material

88 The material analysed is composed of 10 species from the Cerrado vegetation in
 89 Brazil. Table 1 shows the name of the collected species (six samples per species).
 90 More details about the database can be found in da Silva et al. (2015)¹. The
 91 central part of the leaf (including the midrib) was fixed in FAA (Formalin-
 92 Acetic Acid-Alcohol) 70 for 48 hours (Johansen, 1940), then dehydrated in an
 93 ethanol series and embedded in paraffin. Afterwards, 8 μ m-thick cross-sections
 94 were cut, rehydrated and stained with Astra blue and basic fuchsin, dehydrated
 95 and finally mounted with Entellan. The images of midribs were captured with a
 96 10x objective lens, in a trinocular Axio Lab A1 microscope coupled to a digital
 97 AxioCam ICc 1 camera (Zeiss, Germany). After images were captured, the
 98 region containing the midrib was manually separated from the background.

Table 1: Plant species in the database analysed here (six samples per species).

Family	Species
Anacardiaceae	<i>Anacardium humile</i> A. St.-Hil.
Annonaceae	<i>Annona crassiflora</i> Mart.
Aristolochiaceae	<i>Aristolochia galeata</i> Mart. & Zucc.
Bignoniaceae	<i>Arrabidaea brachypoda</i> Bur
Apocynaceae	<i>Aspidosperma subincanum</i> Mart.
Asteraceae	<i>Baccharis salzmannii</i> DC.
Fabaceae	<i>Bauhinia pulchella</i> Benth.
Fabaceae	<i>Bauhinia unguolata</i> L.
Malpighiaceae	<i>Banisteriopsis stellaris</i> (Griseb.) B.Gates
Malpighiaceae	<i>Byrsonima laxiflora</i> Griseb.

99 3 Proposed method

100 The method proposed here consists of 4 steps: pre-processing of the cross-
 101 section, segmentation of v-cells, labelling of layers of v-cells, and extraction of
 102 features from each layer.

¹The database is available for downloading at <https://dataverse.harvard.edu/dataset.xhtml?persistentId=doi:10.7910/DVN/KDZVUM>

103 **3.1 Pre-processing**

104 First, the midrib region of the section is manually selected (by the operator) and
105 separated from the mesophyll region. The midrib image (Figure 1a) was then
106 preprocessed for noise suppression and background illumination by applying the
107 following operation:

- 108 • **Gaussian Blur** - The original colour image is converted to grey-levels,
109 inverted and convolved with a Gaussian filter with radius 5 around each
110 pixel to attenuate some noise and outliers by smoothing (Figure 1b).

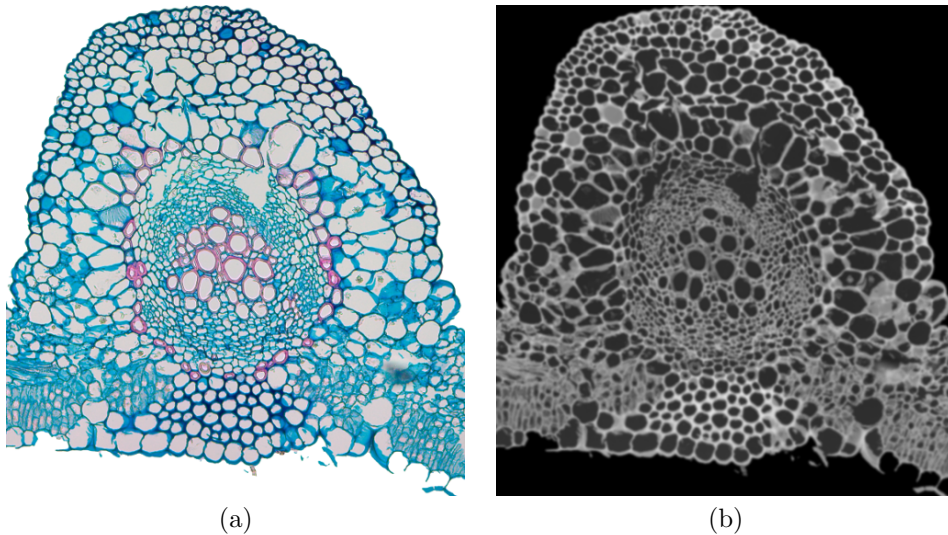


Figure 1: Pre-processing operation. (a) Original image. (b) Grey-level inversion and Gaussian blurring.

111 These steps were carried out in ImageJ². Figure 1 shows the result image
112 after processing.

113 **3.2 Morphological segmentation**

114 The morphological segmentation of grey-level images, used here and presented
115 in Vincent and Dougherty (1994), is based on the watershed transform which
116 relies on detecting 'catchment basins' on the grey-level image, when considering
117 that pixel greyscale values represent the 'height' of the greyscale function. The
118 catchment basins are those regions where imaginary drops of water falling over
119 the image would tend to accumulate. The borders where the different catchment
120 basins meet are called *watershed lines*.

²ImageJ is a public domain program for image processing written by W. Rasband, available at <http://imagej.nih.gov>.

121 Depending on application, the watershed lines are computed from the image
 122 itself, or from its gradient (so the watershed lines tend to be located at the edges
 123 of image objects). Here we exploit a mathematical morphology approach and
 124 use the Beucher's gradient, defined for image I as

$$\text{grad}(I) = (I \oplus B) - (I \ominus B), \quad (1)$$

125 where B is a unitary ball and \oplus and \ominus stand for the morphological dilation and
 126 erosion, respectively.

127 The extraction of watershed lines from the greyscale gradient sometimes
 128 tends to over-segment the image (i.e. produces too many basins). The method
 129 described in Vincent and Dougherty (1994) seeks to avoid this by redefining the
 130 gradient through a morphological grey-level reconstruction driven by a marker
 131 image. Thus given the gradient image $J = \text{grad}(I)$ and the marker image M , a
 132 new reconstructed gradient J' can be computed.

133 First, for every pixel p , J and M are transformed into J^* and M^* by

$$J^*(p) = \begin{cases} h_{min} & \text{if } M(p) = 1 \\ J(p) & \text{otherwise,} \end{cases} \quad (2)$$

134 and

$$M^*(p) = \begin{cases} h_{min} & \text{if } M(p) = 1 \\ h_{max} & \text{otherwise,} \end{cases} \quad (3)$$

135 where h_{min} and h_{max} are chosen such that $\forall p, h_{min} < J(p), h_{max} > J(p)$.

136 At this point, it is important to define the procedure called dual grey-scale
 137 reconstruction. This procedure is not exactly straightforward and so it is ex-
 138 plained next using simpler concepts. The first concept is the n^{th} geodesic dila-
 139 tion $\delta_X^n(Y)$ of a set $Y \subseteq X$. In terms of morphological operations, for $n = 1$:

$$\delta_X^1(Y) = (Y \oplus B) \cap X, \quad (4)$$

140 and $\delta_X^n(Y)$ is defined recursively:

$$\delta_X^n(Y) = \delta_X^1(\delta_X^{n-1}(Y)). \quad (5)$$

141 Based on that, the reconstruction $\rho_X(Y)$ is given by

$$\rho_X(Y) = \lim_{n \rightarrow \infty} \delta_X^n(Y). \quad (6)$$

142 In practice, only a few steps are necessary to achieve the expected reconstruc-
 143 tion.

144 Given two grey-scale images I and J defined over the same domain, as-
 145 suming discrete grey-levels $\{0, 1, \dots, N\}$, and satisfying $\forall p, J(p) \geq I(p)$, the
 146 reconstructed image of I from J is given by

$$\rho_I^*(J)(p) = N - \rho_{N-I}(I - J), \quad (7)$$

147 where ρ is the reconstruction defined by

$$\rho_I(J)(p) = \max\{k \in [0, N] | p \in \rho_{T_k(I)}(T_k(j))\}, \quad (8)$$

148 and $T_k(I)$ is the set of pixels p such that $I(p) \geq k$.

149 Finally, after the above definition, J' is provided by a dual grey-level recon-
150 struction:

$$J'(p) = \rho_{J'}^*(M^*). \quad (9)$$

151 The final step of the segmentation is to extract the watershed lines of J' .

152 More details, rationale and illustrated examples can be found in Vincent
153 and Dougherty (1994). In terms of computational implementation, an imageJ
154 plug-in³ was used, with a tolerance threshold of 10. Figure 2 illustrates how the
v-cells are identified by the method.

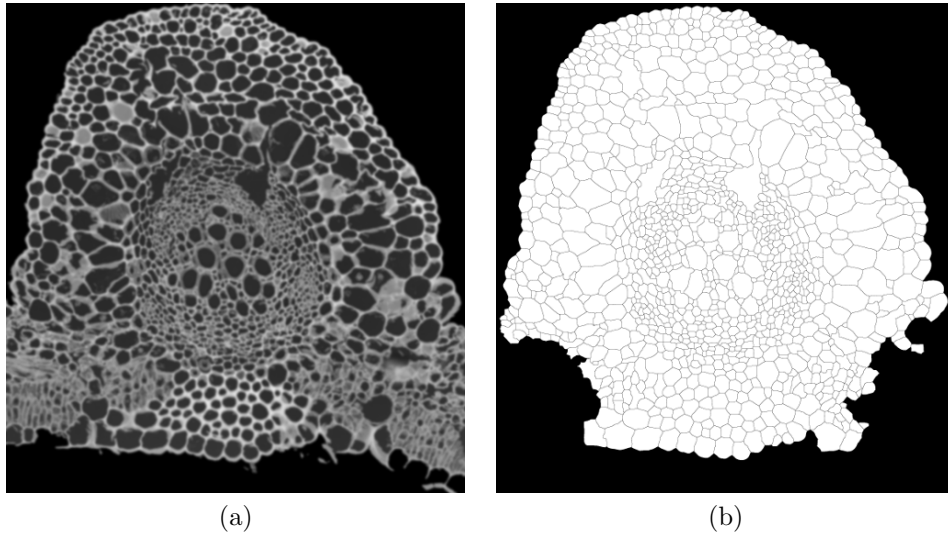


Figure 2: Morphological segmentation of the cross-section cells. (a) Pre-processed image (resulting from Figure 1). (b) Morphological segmentation.

155

156 3.3 Extraction of layers

157 The tissue structure in the cross-sections has a relatively symmetrical arrange-
158 ment, resembling layers of cells and vascular spaces with similar properties (size,
159 shape) across the species studied.

160 The v-cell layers are sets of v-cells that fulfill specific adjacency relations and
161 these can be computed using the algorithm described by Landini and Othman
162 (2003).

³Available at <http://fiji.sc/Morphological.Segmentation>.

163 The first step in this procedure is to identify the outmost external layer.
 164 This is achieved by identifying those v-cells adjacent to the background region.
 165 The v-cells in the segmented cross-section C are merged into C' , by applying a
 166 closing operation:

$$C' = (C \oplus S) \ominus S, \quad (10)$$

167 where S is a 3×3 structuring element.

168 The background is then dilated, which gives rise to a region R of intersection
 169 with the v-cells, computed using

$$R = C \wedge \bar{C}' \oplus S, \quad (11)$$

170 where \bar{C}' is the binary inversion of C' .

171 The first layer is obtained by the binary reconstruction of C from R and
 172 is labelled as 1. Once the first layer has been identified, the remaining layers
 173 are computed by considering the previous layer as the background and applying
 174 two consecutive dilations in Equation 11 (the first one fills the gap between the
 175 v-cells, the second provides the overlap with not-yet-labelled v-cells and which
 176 is used by the reconstruction operation to identify the next adjacent layer). The
 177 layers are increasingly labelled 2, 3, 4, and so on. Here, such labelling procedure
 178 was applied in two directions (from dorsal to ventral surface and vice-versa) and
 179 the minimum of these labels define the layers from the closest free surface of the
 180 leaf.

181 More details and a pseudo-code can be found in Landini and Othman (2003).
 Figure 3 shows the layers identified in the segmented image.

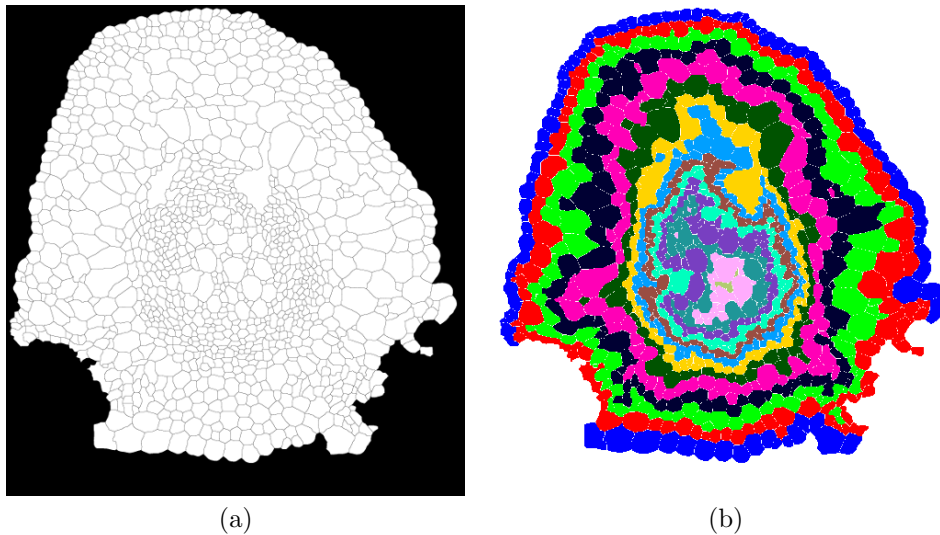


Figure 3: External layers highlighted in different colours over the segmented image. (a) Segmented image (resulting from Figure 2). (b) Extracted layers.

182

183 **3.4 Extraction of layer features**

184 The final step in the proposed method was to compute the features of the v-cells,
 185 according to the layer where they were located.

186 A straightforward and efficient way of representing layer features is to cal-
 187 culate the mean $\mu_L^M(x)$ of the measure M over the cells x in the layer L . A
 188 potential problem of using the mean is that segmentation inaccuracies can result
 189 in very large or small v-cells which are not strictly cellular or vascular struc-
 190 tures, e.g. empty spaces and/or over/under-segmented regions. To minimise
 191 this, outliers with areas 95% larger or smaller than the mean were removed and
 192 $\mu_L^M(x)$ was computed in two steps. In the first an auxiliary mean $m_L^M(x)$ is
 193 computed by

$$m_L^M(x) = \frac{1}{N} \sum_{x \in L} M(x) \quad (12)$$

194 and from that $\mu_L^M(x)$ is obtained through

$$\mu_L^M(x) = \frac{1}{N} \sum_{x \in L'} M(x), \quad (13)$$

195 where $L' = \{x \mid \|M(x) - m_L^M(x)\| < 0.95 \sup(\|M(x) - m_L^M(x)\|)\}$.

196 For M , 25 different measures were employed. They were inspired by (Landini,
 197 2006, 2008) and are listed in Table 2. A complete description of each measure
 can be found in Landini (2006, 2008).

Table 2: Measures M computed for each v-cell.

Perimeter	PerimEquivD
Area	EquivEllipseAr
MinR	Compactness
MaxR	Solidity
Feret	Concavity
Breadth	Convexity
CHull	Shape
CArea	RFactor
MBCRadius	ModRatio
AspRatio	Spericity
Circ	ArBBox
Roundness	Rectang
AreaEquivD	

198 Finally, all the values of μ_L^M , for each layer L and each measure M , were
 199 concatenated to create a vector of features. This results in a large set of features,
 200 depending on the number of layers and the number of measures considered in
 201 each layer. To address this and produce results comparable to other classifica-
 202 tion methods, the concatenated features were submitted to a Karhunen-Loève
 203 transform (also called Principal Component Analysis or PCA) to reduce the
 204

205 data dimensionality. The most significant scores were used in the next machine
 206 learning step. Figure 4 shows a simple diagram of the method described for the
 analysis of a leaf cross-section.

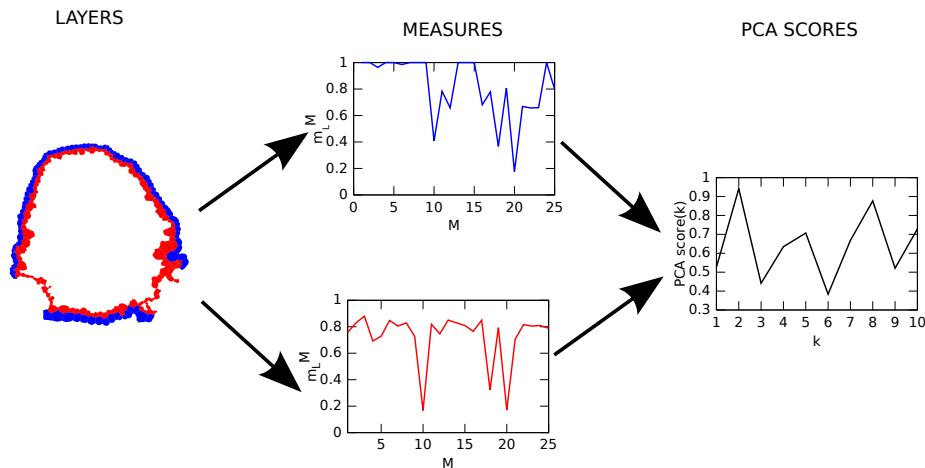


Figure 4: A basic diagram of the proposed method. From left to right, the
 layered cross-section, the average measures estimated over two layers and the
 resulting concatenation after Karhunen-Loève reduction.

207

208 3.5 Assessment

209 The categorization of species was carried out by means of a learning machine
 210 method using the PCA scores as input. A cross-validation strategy was em-
 211 ployed, where the samples were split into training and testing sets, following a
 212 10-fold procedure. This consisted of dividing the samples into 10 subsets with
 213 similar sizes and in each round nine subsets were used for training and the re-
 214 maining one for testing (as described in Duda and Hart (1973)). The categoriza-
 215 tion was finally accomplished by a Linear Discriminant Analysis (LDA) (Duda
 216 and Hart, 1973), following similar approaches used in other reported plant image
 217 analyses (Backes et al., 2009; Florindo et al., 2014).

218 We compared our results to those from shape-based methods previously re-
 219 ported in the literature, namely, Multiscale Fractals (Bruno et al., 2008) (using
 220 the entire multiscale curve), Invariant Moments (Hu, 1962) (7 moments) as well
 221 as a classification based on the measures computed over the v-cells (irrespective
 222 of the layers where they were located). Those methods were implemented fol-
 223 lowing the published descriptions to analyse the contour of the v-cells. The mul-
 224 tiscale fractal descriptors were computed on the shapes of v-cells as the object
 225 of interest, whereas the moment descriptors were averaged over all the v-cells.
 226 To make results comparable across methods in terms of descriptor numbers, the
 227 extracted feature vectors were submitted to the Karhunen-Loève transform and
 228 the first n scores (up to a maximum of 100) were used in the assessment.

229 4 Results and discussion

230 Table 3 shows the success rates obtained by the proposed method and other
231 published approaches. Our method outperforms the direct use of v-cells and
232 all the others by at least 5%, showing how the architectural analysis is able
233 to provide additional information on the structural morphology of leaf cross-
234 section. We also investigated whether unbalanced sized groups of species in the
235 database made significant differences to the results. We repeated the analysis
236 using all the samples in the database (total $n = 96$, instead of six samples per
237 species, $n = 60$) and found that the correct rate of identification for the proposed
238 method was practically the same (91.8%) and this result was also higher than
for the “Cells” approach (87.3%).

Table 3: Correctness rate obtained by the proposed method, in comparison with other shape-based approaches in the literature.

Method	Correctness rate (%)	Number of features
Invariant moments	46.7±0.2	4
Multiscale fractal	68.3±0.2	3
Cells	86.7±0.1	19
Proposed method	91.7±0.1	12

239 The results show that the analysis of midrib architecture is useful to de-
240 scribe its complex structure in cross-sections and highlights the importance of
241 micro-anatomy in plant taxonomy, in particular how the cellular structures are
242 assembled following a characteristic order and pattern, which appears to be
243 characteristic and conserved in samples of the same species, but statistically
244 different across species.

245 There are still some challenges that need to be resolved to fully automate
246 this type of analysis. Two of these are the spatial orientation of the specimen
247 within the image frame and the identification of the midrib section (the region
248 of interest), which currently require operator input.

250 5 Conclusions

251 This work presented a new type of analysis for the identification of plant species
252 based on microscopical images of midrib cross-sections. Instead of the classical
253 shape-based analysis focused on leaf contours or individual cells, we have the
254 partitioning of the midrib cross-sections into histologically relevant structures
255 (cell and vascular spaces) and their spatial organization (layers). This provided
256 a level of description that machine learning procedures were able to exploit for
257 the identification of plant species.

258 The performance of the method was tested using a database of plants from
259 the Brazilian *flora* (da Silva et al., 2015) and compared with previous approaches
260 published in the literature. Our method achieved a success rate of 91.7% over a
261 set of 10 plant species. This is an encouraging result, which is higher than the

262 performance of other computational approaches to this problem. Furthermore,
263 this also gives some idea of how a layer-wise analysis can improve a shape-based
264 analysis of this type of material, suggesting a more in-depth investigation in the
265 future.

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