

1 Quantitative Estimation of Bioturbation Based on Digital 2 Image Analysis

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16

17 **Abstract**

18 Quantitative determination of modification of primary sediment features, by the
19 activity of organisms (i.e., bioturbation) is essential in geosciences. Some methods
20 proposed since the 60's are mainly based on visual or subjective determinations. The
21 first semiquantitative evaluations of the bioturbation index, ichnofabric index, or the
22 amount of bioturbation were attempted, in the best cases using a series of flashcards
23 designed in different situations. Recently, more effective methods involve the use of
24 analytical and computational methods such as X-rays, magnetic resonance imaging or

25 computed tomography; these methods are complex and often expensive. This paper
26 presents a compilation of different methods, using Adobe® Photoshop® software CS6,
27 for digital estimation that are a part of the IDIAP (Ichnological Digital Analysis Images
28 Package), which is inexpensive alternative to recently proposed methods, easy to use,
29 and especially recommended for core samples. The different methods —“Similar Pixel
30 Selection Method (SPSM)”, “Magic Wand Method (MWM)” and the “Color Range
31 Selection Method (CRSM)”— entail advantages and disadvantages depending on the
32 sediment (e.g., composition, color, texture, porosity, etc.) and ichnological features (size
33 of traces, infilling material, burrow wall, etc.). The IDIAP provides an estimation of the
34 amount of trace fossils produced by a particular ichnotaxon, by a whole ichnocoenosis
35 or even for a complete ichnofabric. We recommend the application of the complete
36 IDIAP to a given case study, followed by selection of the most appropriate method. The
37 IDIAP was applied to core material recovered from the IODP Expedition 339, enabling
38 us, for the first time, to arrive at a quantitative estimation of the discrete trace fossil
39 assemblage in core samples.

40

41 **Keywords:** Bioturbation, digital images, quantitative methods, marine core deposits,
42 Integrated Ocean Drilling Program, Expedition 339, Site U1385

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44 **1. Introduction**

45

46 The beginning of the twenty-first century has witnessed a rapid growth in
47 ichnological research, which has become relevant in a wide range of fields, e.g,
48 palaeobiology, palaeoecology, biostratigraphy, sedimentology, and most recently
49 reservoir characterization, involving biologists, palaeontologists and sedimentologists.

50 In some of these disciplines, the quantitative determination of a modification of primary
51 sedimentary features by bioturbation can prove fundamental. Such is the case of
52 reservoir and aquifer characterization, and the impact of trace fossils and their
53 associated ichnofabrics on fluid-flow properties, including sediment permeability and
54 porosity (Cunningham et al., 2013; Gingras et al., 2013).

55 The intensity of bioturbation is characterized by the use of semi-quantitative
56 index schemes (i.e., Marenco and Bottjer, 2011; Ekdale et al., 2012; Knaust, 2012), the
57 most common ones being the “Bioturbation Index” (BI) (Reineck, 1963) and
58 “Ichnofabric Indices” (ii) (Droser and Bottjer, 1986). The “Bioturbation index” of
59 Reineck (1963), later revised by Taylor and Goldring (1993), was initially applied to
60 box cores and has a scale from Bioturbation Index = 0 (no bioturbation) to 6
61 (completely bioturbated). In turn, the “ichnofabric indices” of Droser and Bottjer
62 (1986), widely used in ichnological research, range from ichnofabric index = 1 (no
63 bioturbation) to ichnofabric index = 6 (sediment nearly or totally homogenized), and
64 rely on the use of flash cards for an easy visual assessment of the intensity of
65 bioturbation. A modification of the ichnofabric indices, the “Bedding Plane Horizontal
66 Index” (BPHI), was proposed by Miller and Smail (1997) for the degree of bioturbation
67 on originally horizontal planes. All three methods are easy to apply and inexpensive,
68 however, they are mainly based on visual observation, using grades or scores that
69 represent different ranges of percentage, thus bearing the possibility of a subjective
70 inaccuracy for estimation of bioturbation intensity.

71 In the wake of the early semi-quantitative approaches of Reineck (1963), Droser
72 and Bottjer (1986) and Taylor and Goldring (1993), more objective methods appeared
73 that involved either grid-based techniques, computer-aided image analysis or advanced
74 image technology (Marenco and Bottjer, 2011). Grid-based methods, which can be used

75 with different scales of grids, are particularly useful for estimating the quantity of
76 bioturbation on bedding planes or on vertical cross-sections (Heard and Pickering,
77 2008; Marengo and Bottjer, 2010). They are inexpensive and simple to perform,
78 requiring little specialized equipment, but they are time-consuming and accuracy
79 depends on grid and trace sizes. Computer-aided image analysis has been used to
80 improve the visibility of complex ichnofabrics in photographs (Magwood and Ekdale,
81 1994; Dorador et al., 2013), and to estimate the amount of bioturbation (Francus, 2001;
82 Löwemark, 2003). Computer-aided image analysis is appropriate for specialized
83 applications, such as thin-sections or x-radiographs, and sometimes calls for the use of
84 Scanning Electron Microscopy; a further limitation is the reduced field of view. Image
85 techniques, such as x-radiography (e.g., Löwemark, 2003; Marengo and Bottjer, 2008),
86 computed tomography (e.g., Fu et al., 1994; Dufour et al., 2005), and magnetic
87 resonance imaging (e.g., Gingras et al., 2002), have been recently applied to acquire
88 information on trace fossils and ichnofabrics, but scarcely applied for estimation of the
89 amount of bioturbation (e.g., Dufour et al., 2005). Except for x-radiographs, imaging
90 techniques as computed tomography and magnetic resonance imaging are complex and
91 very expensive.

92 This paper presents a novel, easy to use, and inexpensive package (IDIAP;
93 Ichnological Digital Analysis Images Package) of different methods for digital
94 estimation of discrete trace fossil assemblage in digital images using Adobe®
95 Photoshop® software CS6. The method can be applied on images of samples acquired
96 from outcrops and cores, but is especially appropriate for the latter due to the
97 specific features of cores (i.e., limited size, restricted surface, among others) make
98 ichnological research difficult. The package consists of three different methods enabling
99 its application in a great variety of material having different sediment and ichnological

100 features. We demonstrate the usefulness of the IDIAP by applying it to core material
101 from the IODP Expedition 339 (Fig. 1).

102

103 **2. The Ichnological Digital Analysis Image Package (IDIAP)**

104

105 The Ichnological Digital Analysis Images Package (IDIAP) integrates three
106 methodologies of digital image analyses: the “Similar Pixel Selection Method (SPSM)”,
107 the “Magic Wand Method (MWM)” and the “Color Range Selection Method (CRSM)”
108 (Fig. 2). IDIAP provides a quantitative assessment of the percentage of area occupied
109 by discrete trace fossils in core material. Depending on the particular sediment and its
110 ichnological features, any one of the three methods may be considered more
111 appropriate, or an integrative approach using all of them may be best.

112

113 *2.1. Similar Pixel Selection Method (SPSM)*

114

115 This method allows finding pixels on the full image with values in the range of
116 those pixels registered in a previously selected particular area, which correspond to the
117 studied trace fossil. It is useful when objects need to be selected with a variable range of
118 colors because an area with a certain number of pixels is chosen. In cases where objects
119 cannot be defined by only one pixel color, this method has a major advantage. A
120 representative area within the desired object is selected using any Photoshop selection
121 tool. After that, click ‘Select’ in the menu bar and choose ‘Similar’. The method’s great
122 disadvantage, however, is that the selection sensitivity cannot be modified; it is not
123 useful if the difference between pixels is low. Further, it can only be executed once; if
124 there are different objects of different colors, they cannot all be selected. The SPSM

125 may be considered as a more complete and easier method than the one presented by
126 Honeycutt and Plotnick (2008). The method used by Honeycutt and Plotnick (2008) is
127 an automatic quantitative method to determine the Bioturbation Index using the
128 complex software Matlab 7.1 ® from digital images, based on gray-level matrices (one
129 value per pixel) while the SPSM uses an easier software based on digital color images
130 (three values per pixel).

131

132

133 *2.2. Magic Wand Method (MWM)*

134

135 This method alludes to the ‘Magic Wand tool’ used by Coimbra and Olóriz
136 (2012) for the manual selection of objects, this tool selects pixel nearby (by default)
137 having similar variations of color of the previously selected pixel. The maximum
138 percentage difference between the reference pixel and the selected pixels is defined by a
139 parameter called ‘Tolerance’. This method has been used to derive percentage
140 estimations of bioclasts and other applications, but not to ichnological data, based on
141 pixel counting using Adobe® Photoshop® software CS2 (e.g. Perring et al., 2004;
142 Johansson et al., 2008). It can be time-consuming, especially if there are small objects
143 in the image, calling for clicks on each object to be selected. However, it affords the
144 great advantage of multiple executions. To proceed, the ‘Magic Wand Tool’ is selected
145 and one representative pixel within each object is chosen. The sensitivity is controlled
146 by ‘Tolerance’ that can be modified in the menu bar until obtaining the desired
147 selection. To add more than one object, simply click on the ‘Add to selection’ option in
148 the menu bar and click on a new object. Especially, when objects are large and possess
149 more than one color this method is useful. Additionally, the method can be modified to

150 be applied to the complete given image. It even works with smaller objects when these
151 clearly differ from the background.

152 The pixels selected can be manually modified with different tools if there is any
153 object whose selection is not possible with one of these automatic methods.

154

155 *2.3. Color Range Selection Method (CRSM)*

156

157 This method is based on the localization of pixels in the whole image that are
158 similar to only one manually selected. It shares a great advantage with the SPSM: the
159 selection extends over the full image, but in the case of the CRSM only one
160 representative pixel can be selected, while in the SPSM all pixels registered in the
161 selected particular area are considered by the method. The method allows choosing a
162 selection or a desired pixel range with the modification of a parameter called
163 ‘Fuzziness’. This method is the quickest and most useful when there are a lot of small
164 objects of the same or similar color. In order to execute it choose ‘Select’ from the
165 menu bar and click on ‘Color range...’, and a window opens. You may define the
166 reference color to extend the selection, but the best and easiest way to select similar
167 objects is to choose ‘Sampled Colors’. Under this option, the cursor is a sampled color,
168 and you have to click on a representative pixel of the object color. Then, a selection
169 takes place, and it can be viewed in a little window so that the fuzziness parameter can
170 be modified until all objects are selected, and then you click on ‘OK’. This method only
171 can be executed once.

172

173 **3. Results From The Expedition 339 Core Material**

174

175 The IDIAP was applied to IODP Expedition 339 cores (Expedition 339, 2013;
176 Hernández-Molina et al., 2013). Some intervals with trace fossils were selected from the
177 core material of site 1385 (southwestern Iberian margin; 37°34.285'N, 10°7.562'W;
178 Hodell et al., 2013). The sediments recovered in the core are dominated by Pleistocene
179 hemipelagic mud- and claystones. No primary sedimentary structures were observed.
180 The discrete trace fossil assemblages include *Zoophycos*, *Chondrites* and *Planolites* as
181 the dominant ichnotaxa (Expedition 339, 2013). The section of this site is typical of a
182 hemipelagic continental margin succession under normal marine conditions (Expedition
183 339, 2013; Hernández-Molina et al., 2013; Hodell et al., 2013). The principal colors of
184 lithologies range from gray to greenish gray, impeding trace fossil distinction and
185 identification at first glance by digital images. To facilitate the recognition of distinct
186 trace fossils, selected images were slightly treated beforehand to identify and select the
187 trace fossils as follows: Each image was modified, using Photoshop, by increasing its
188 contrast up to '100' to improve quality. After application of the IDIAP, selection of
189 discrete trace fossils must be checked visually and in some cases slightly modified by
190 hand. This modification is done because sometimes there are selected pixels that are
191 unrelated to trace fossils but they are interpreted as traces by the software. Modification
192 was done using 'Elliptical or Rectangular Marquee Tools', which make it possible to
193 add an area ('Add to selection') or diminish an area ('Extract to selection'). These
194 modifications were also used when cross-cutting relationships existed (Figs 4, 5;
195 examples B and C), to distinguish in the estimation between those areas occupied by the
196 first generation of traces and that by the second generation.

197 After selection, every ichnotaxon was painted with different colors applying the
198 "Paint Bucket Tool" to emphasize the selection and facilitate the percentage estimation.
199 The number of selected pixels is shown in the 'Histogram' window; pixel selection

200 value (P_s) should be compared with the number of pixels in the total area of the image
201 (P_I) and converted in percentage. (Note: the histogram does not show cached data). The
202 whole procedure was executed more than one time in the same picture and the variation
203 in the area occupied by traces was lower than 5%. This procedure has been summarized
204 in a flow-chart (Fig. 2). To test the IDIAP, three examples were selected, representing
205 different cases of abundance, diversity and complexity of discrete trace-fossil
206 assemblages:

207

208 Our first example A (Fig. 3), located in 1385E-13H-6A, 38-42 cm, shows scarce
209 *Thalassinoides*-like and *Phycosiphon*, without cross-cutting relationships. The three
210 methods were applied with different results. Owing to the lack of contrast between
211 traces and sediment, the SPSM gave wrong selections and therefore did not only select
212 pixels belonging to the trace fossils but also of the surrounding sediment. The CRSM on
213 the other hand, selected not all pixels belonging to the *Thalassinoides*-like pixels. The
214 best CRSM result was obtained with a fuzziness value of 11. Visually, the MWM was
215 superior although every trace had to be clicked on. The best tolerance values used were
216 3-5, due to slight differences between *Phycosiphon* and the surrounding pixels. Thus,
217 the best estimation overall was obtained using MWM: the bioturbated percentage value
218 corresponding to trace fossils was roughly 5% (4.3% *Thalassinoides*-like, 0.8%
219 *Phycosiphon*).

220

221 The example B figured in Fig. 4, located in 1385A-5H-5, approximately 126-
222 132 cm, shows two phases of colonization with a more abundant first generation of
223 traces with *Thalassinoides*-like, *Thalassinoides* and *Planolites*, crosscut by a less
224 abundant second generation of *Chondrites*. SPSM and CRSM results were very similar.

225 The first gave 56% area of trace fossils, and CRSM, which was applied with a fuzziness
226 value of 20, gave 49%. On the other hand, the result from the MWM was 37% area of
227 trace fossils with applied tolerance values of 5-10. Yet for this example, full image
228 methods prove more successful visually because there were many small traces that were
229 difficult to identify and using MWM would be very time-consuming. The percentage of
230 area corresponding to trace fossils was around 52%, according to SPSM and CRSM
231 values; from these 52%, 47.3% were produced by the first generation of traces (23%
232 *Thalassinoides*-like, 22% *Thalassinoides* and 2.3% *Planolites*) and 5.3% by the second
233 generation (*Chondrites*).

234

235 From Hole 1385E, Core 13H-4A, 117-125 cm approximately, example C (Fig.
236 5) shows two phases of colonization with a comparatively less abundant first generation
237 with *Planolites* and *Thalassinoides*, crosscut by a more abundant second generation of
238 *Zoophycos*. We used the “Magic Wand Method” with a tolerance value of “3”. When
239 SPSM was used, most of *Zoophycos*- and *Planolites*-pixels were selected but pixels
240 corresponding to diffuse *Thalassinoides* were not. Several non-trace pixels were
241 selected, so the 19% value obtained is not reflecting the true grade of bioturbation
242 corresponding to trace fossils. CRSM, with a fuzziness value of 92, selected the most
243 trace pixels (except some *Thalassinoides* pixels) but also selected a lot of non-trace
244 pixels, thus giving around 51% area of trace fossils which is much, higher than the real
245 value. The best results were achieved with the MWM, because *Thalassinoides* could be
246 selected using the ‘Magic Wand Tool’ with a tolerance value of 3 and non-trace pixels
247 were not selected. The real percentage of area corresponding to trace fossils, then, was
248 about 17%, as estimated by the MWM: 3% for the first generation of traces (2.7%
249 *Thalassinoides*, and 0.6% *Planolites*) and 14% for the second one (*Zoophycos*).

250

251 **4. Discussion And Conclusions**

252

253 The IDIAP represents a novel, easy to use, and inexpensive package of digital
254 imaging methodologies for quantitative estimation of the percentage of discrete trace
255 fossils in core material. The IDIAP allows for quantitative estimation of area occupied
256 by particular ichnotaxa, the whole ichnocoenosis, and even a complete ichnofabric.
257 Each one of the three methods —“Similar Pixel Selection Method (SPSM)”, “Magic
258 Wand Method (MWM)” and the “Color Range Selection Method (CRSM)” — shows
259 advantages and disadvantages depending on the sedimentological and ichnological
260 features of the core material, as demonstrated by the analyses conducted on cores from
261 the IODP Expedition 339.

262 The SPSM was most successful in example B (Fig. 4), where numerous small
263 traces are clearly differentiated from the host material. In cases involving poorly
264 differentiated traces with respect to the host material (e.g., A; Fig. 3), this method
265 proves inconsistent and should not be applied, as it selected a full image. However, this
266 method is the quickest because it is not necessary to define parameters. Hence it is
267 especially appropriate for situations of abundant small discrete traces possessing large
268 differences in pixel color in comparison to the surrounding sediment, making it possible
269 to choose the vast majority of traces at the same time. It would not be the method of
270 choice in situations with minor differences between pixels, because its ‘sensitivity’
271 cannot be modified and it would select too many pixels. Furthermore, this method does
272 not allow for adding another selection. For the cores from site 1385 of the IODP
273 Expedition 339, consisting of hemipelagic sediments with scarce differences in pixels
274 between the trace infilling and the surrounding material, this method is inappropriate; In

275 such cases the MWM and the CRSM should be used, because these methods allow the
276 necessary modifications of the sensitivity.

277 The MWM proves to be an interesting option that allows the selection of each
278 distinct trace fossil by clicking on it. Of course when dealing with small and abundant
279 discrete traces, such as *Chondrites* (example B; Fig. 4), this is cumbersome, however, it
280 is useful when dealing with larger traces like *Thalassinoides* or *Thalassinoides*-like
281 (examples A and C; Figs 3, 5). In case of only a few traces in a given picture, it is a
282 quick method, requiring just one click per trace fossil. The great advantage is that the
283 sensitivity can be modified through ‘Tolerance’. This is a useful parameter in the
284 analyzed cores, as differences between pixels are so low that the right selection has to
285 be made by setting the tolerance value very low. The MWM gave the best results in
286 situations with large to medium size trace fossils (more than 5mm) infilled by material
287 similar in color to the host sediment.

288 The CRSM gave results similar to those obtained with the SPSM. It was most
289 useful for our example B (Fig. 4) to control the sensitivity by using the fuzziness
290 parameter. Given this advantage, we recommend the CRSM for situations where small
291 traces are present whose fills have a sufficient color difference in comparison to the
292 surrounding sediment.

293 A complete application of the IDIAP is advisable in any case study, each of the
294 three methodologies having its pros and cons. For images with small discrete traces
295 with similar colors, the SPSM and the CRSM would be best as the selection is easily
296 extended to the full image and they are quicker than the MWM. The SPSM, as the
297 quickest of the three methods, does not allow for modification of the sensitivity, so the
298 color of the infilling material of the trace must be clearly distinguished from the
299 background color. If this difference is not sufficient enough, or the traces have no

300 representative color, the CRSM is the best option because more than one pixel can be
301 selected and the sensitivity for the selection made can be controlled. In situations where
302 there are scarce but large-scale traces, or traces with different colors, the MWM is
303 recommended despite being more time-consuming, again depending on the differences
304 in color between the infilling material and the host sediment.

305

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398

399 **Figure Captions**

400

401 **Fig. 1.** Geographical map showing the west Iberian Margin with location of site site
402 U1385 from the IODP Expedition 339.

403

404 **Fig. 2.** Flow chart showing sequential of processes conforming IDIAP (Ichnological
405 Digital Analysis Image Package) methodology applied to the Expedition 339 core
406 material (see text for explanation). SPSM: Similar Pixel Selection Method, MWM:
407 Magic Wand Method and CRSM: Color Range Selection Method.

408

409 **Fig. 3.** Example A, showing the scarce discrete trace fossils *Thalassinoides*-like (*Th*-1)
410 and *Phycosiphon* (*Phy*), without cross-cutting relationships. From top to bottom;
411 original image without any treatment, “Similar Pixel Selection Method (SPSM)”,
412 “Magic Wand Method (MWM)”, “Color Range Selection Method (CRSM)”, and image
413 showing different colors for the differentiated ichnotaxa.

414

415 **Fig. 4.** Example B, showing two phases of colonization with a comparatively more
416 abundant first generation of trace fossils composed by *Thalassinoides*-like (*Th*-1),
417 *Thalassinoides* (*Th*) and *Planolites* (*Pl*) crosscut by a less abundant second generation
418 consisting of *Chondrites* (*Ch*). Note: Legend as in Figure 3.

419

420 **Fig. 5.** Example C, showing two phases of colonization with a comparatively less
421 abundant first generation of trace fossils composed by *Planolites* (*Pl*) and

422 *Thalassinoides* (*Th*), crosscut by a more abundant second generation consisting of
423 *Zoophycos* (*Zo*). Note: Legend as in Figure 3.

Figure1
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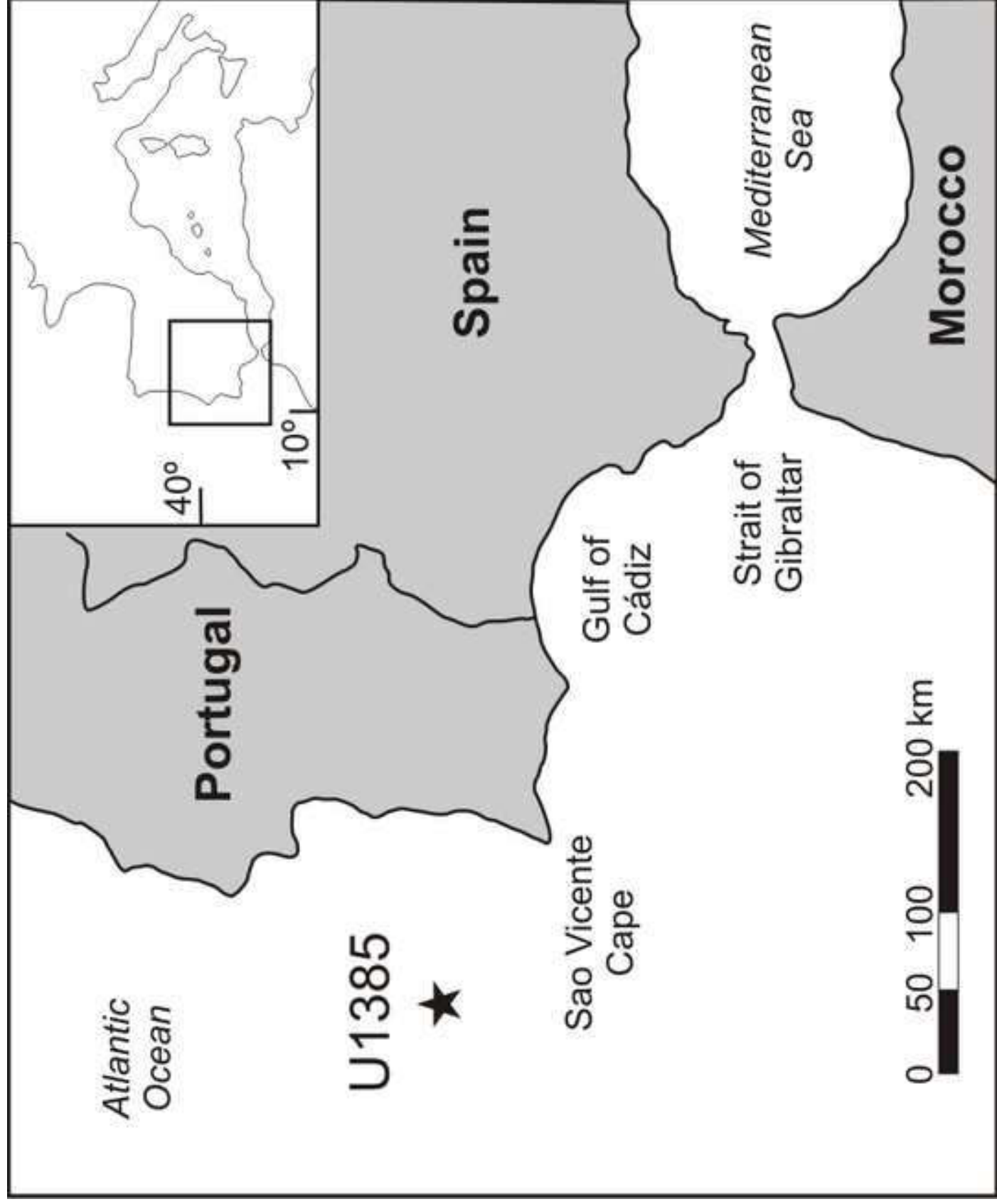


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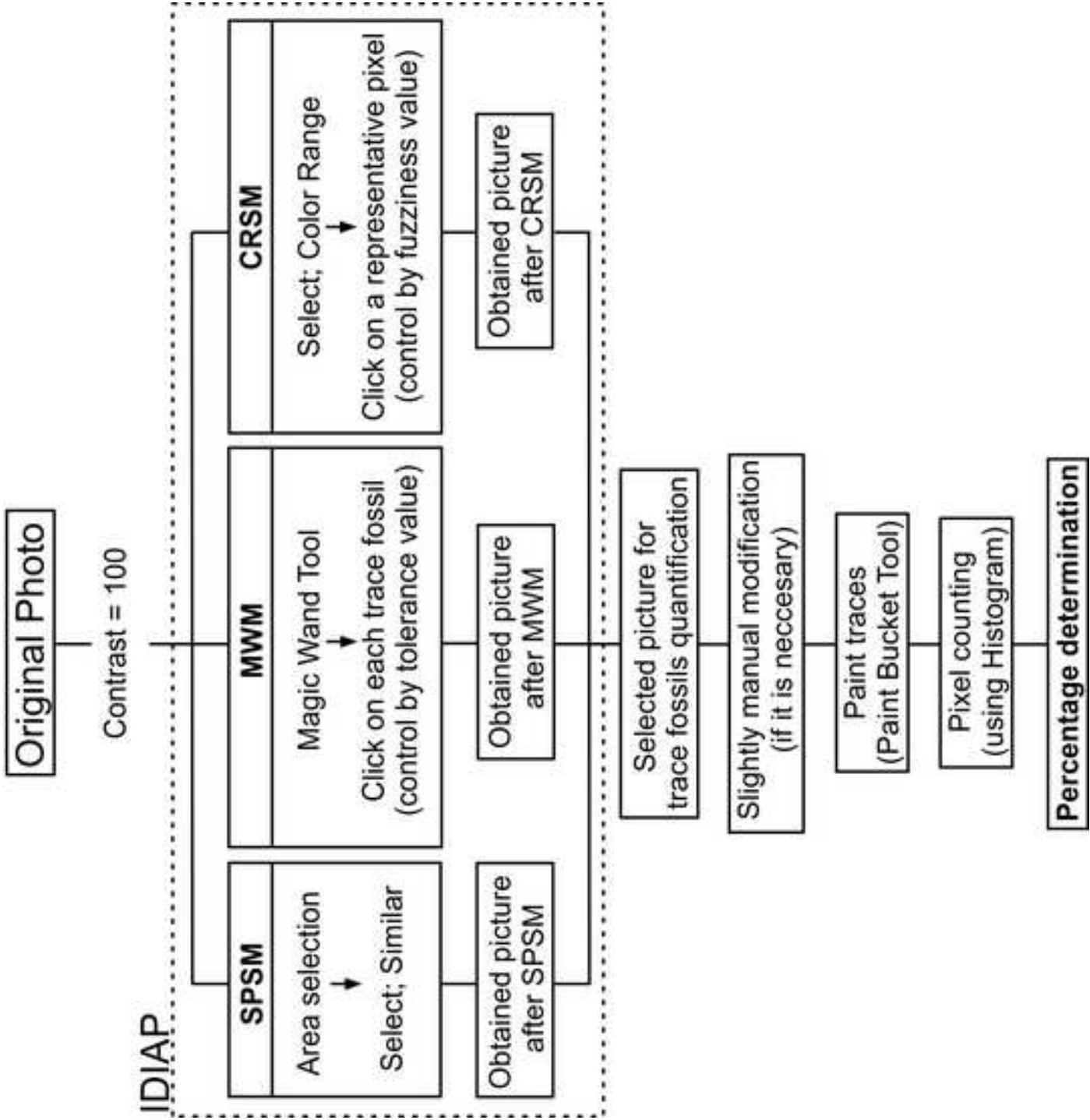


Figure3
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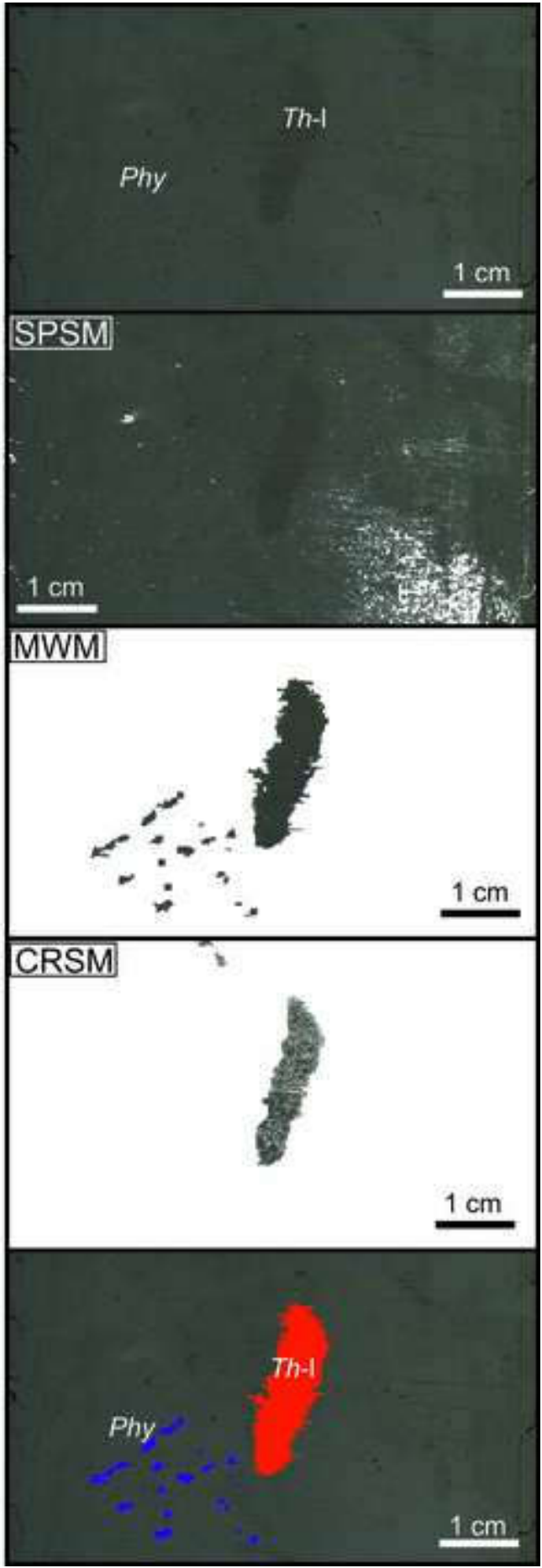


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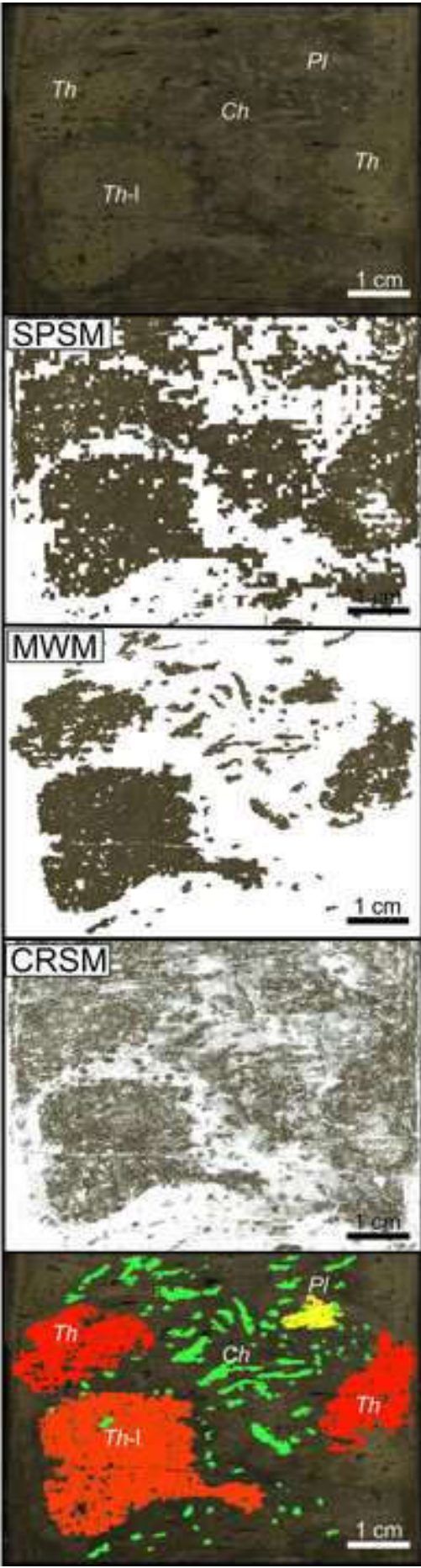


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