Introducing Fiji and ICY image processing techniques in ichnological research as a tool for sedimentary basin analysis

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Abstract

In recent years, image treatment has been appraised as a very powerful tool to facilitate ichnological analysis, especially in marine cores of modern sediments, supporting the determination of certain ichnological features. However, it is still a new approach and detailed research is necessary to encounter a faster and more efficient method. The present study focuses on two image processing techniques, Fiji and ICY, and their comparison with a refined version of the well-established high-resolution image treatment. Strengths and weaknesses of the methodologies for the determination of three main features were explored: i) visibility of trace fossils; ii) quantification of the percentage of bioturbated surface, and iii) penetration depth estimation. Refined high-resolution image treatment gives the best results for enhanced visibility of trace fossils, whereas Fiji is found to be a sound and rapid option. One disadvantage shared by Fiji and ICY is the binary character of the produced images, which may impede later ichnotaxonomical differentiation. Both Fiji and ICY (+ Fiji) are rapid alternatives for quantifying the bulk amount of bioturbated surface. The Magic Wand Method (+ RefineEdge), based on high-resolution image treatment, provides good results regardless of the contrast of the images, and it additionally allows for a more detailed quantification. The semi-automatic character of ICY favors quick estimation of penetration depth and facilitates differentiation between distinct tracemaker communities, based on a rapid quantification of pixel values. Thus, Fiji and ICY methods offer good results and are much less time-consuming than high-resolution image treatment. They are proposed as faster alternatives for the estimation of ichnological features, especially useful at the beginning stages of research, when a large number of samples must be analyzed.

Keywords: Image treatment methods, high-resolution images, degree of bioturbation, penetration depth, basin research.

1. Introduction

The study of trace fossils has been documented as a very useful tool in several Earth Sciences disciplines including palaeontology, sedimentology, marine geology or palaeoceanography, and proven key in sedimentary basin research(e.g., Buatois and Mángano, 2011; Knaust and Bromley, 2012). Trace fossils record the behavior of tracemakers in response to the environment, providing valuable information regarding paleoenvironmental conditions. Ecological and depositional parameters affecting the marine realm (i.e., oxygenation, nutrients, hydrodynamic energy, rate of sedimentation, etc.) can be addressed through a detailed ichnological analysis. Thus, ichnology is a valuable proxy for approaching marine processes or deposits. Most of these ichnological advances have entailed the application of two main paradigms: the ichnofabric approach (McIlroy, 2004; Buatois and Mángano, 2011; Ekdale et al., 2012) and the ichnofacies model (Buatois and Mángano, 2011; MacEachern et al., 2012), both based on a detailed characterization of ichnological properties (i.e., trace fossil assemblage, degree of bioturbation, among others). Occasionally, these properties are difficult to determine due to a poor visibility of the trace fossils in the studied samples. Against this background, in recent years, high-resolution image treatment has been successfully applied for the ichnological analysis of marine cores from modern deposits, proving useful for ichnofabric characterization and ichnofacies differentiation (see Dorador and Rodríguez-Tovar, 2018 for a recent review of the methodology).

Image treatment allows for a better visualization of biogenic structures, thereby making it easier to identify ichnological properties such as morphology, degree of bioturbation or penetration depth, which are of special relevance in applied ichnology (e.g., Bednarz and McIlroy, 2009; Dorador et al., 2014a, b; Rodríguez-Tovar and Dorador, 2014, 2015; Timmer et al., 2016, among others). The use of high-resolution image treatment also contributes to studies surrounding paleoenvironmental developments (e.g., Rodríguez-Tovar and Dorador, 2014; Rodríguez-Tovar et al., 2015a, b; Dorador and Rodríguez-Tovar, 2016a; Dorador et al., 2016) and provides information for sedimentary basin research (e.g., Alonso et al., 2016; Dorador and Rodríguez-Tovar, 2016b; Takashimizu et al., 2016), supported by the ichnological approach. So far, these techniques have been mainly applied on marine cores from modern deposits. However, Dorador and Rodríguez-Tovar (2018) recently showed that image treatment might be an adequate method for analyzing lithified cores and rock samples. While its usefulness has been clearly demonstrated, the application of high-resolution image treatment takes time, a plain fact that encourages further exploration of this approach to find the most efficient and less time-consuming methods. With this goal in mind, two software packages widely used in biological and medical research were selected to test and compare their performance: Fiji, as an extension of the ImageJ software; and ICY software.

Fiji software (Schindelin et al., 2012) is an extension of the well-known open-source software ImageJ, commonly but not exclusively applied in biological and medical studies (Schneider et al., 2012 and references therein), and less frequently in geological research (e.g., Grove and Jerran, 2011; Goldstein et al., 2017). ImageJ has been punctually used in ichnological analysis to enhance visibility of certain image attributes, to estimate bioturbated surface and to make shape/length measurements (Francus, 2001; Nicolo et al., 2010; Lauridsen et al., 2011; Curth et al., 2014). Fiji has already been applied in neo-ichnological analysis for format conversion in reconstructed volumes obtained by computed tomography of invertebrate burrow systems (Hale et al., 2015), and recently to estimate the degree of bioturbation (Miguez-Salas and Rodríguez-Tovar, 2019). This method holds great potential for ichnological studies because its toolbox features diverse tools and filters for the measurement of specific paleontological parameters (i.e., shape/length of bones; Iepure et al., 2012; Jarrett, 2016; O'Connor et al., 2018).

Whereas ICY software has been applied in cell biology studies for tracking, extracting or actively delimiting particles/cells (e.g., de Chaumont et al., 2011; Meijering et al., 2012; Montagnac et al., 2013), it has not been previously applied to geological research. The present research marks the first documented testing of its usefulness in the realm of ichnological aspects.

The aim of this paper is to examine two image processing techniques, Fiji and ICY, to approach a fast and useful method for the early research stages of ichnological studies in which numerous samples and images are examined. Then, the results obtained with the application of these two techniques are compared with a slightly modified version of a previously applied high-resolution image treatment (recent review in Dorador and Rodríguez-Tovar, 2018). In light of their comparison, the strengths and weaknesses of each procedure are characterized, outlining the most efficient approach for applied ichnology in sedimentary basin research.

2. Methods and materials

Two image treatment methods applied to ichnology—Fiji and ICY— plus the refined version of high-resolution image treatment were assessed for their usefulness in ichnological analysis. The focus was on: (1) enhanced trace fossil visualization; (2) quantification of bioturbated surface corresponding to discrete trace fossils; and (3) minimum penetration depth.

2.1. Fiji

Fiji software offers a wide range of plugins for enhancing image visibility, similar to the image adjustments of Adobe Photoshop (e.g., *brightness*, *hue*, *saturation*, *levels*). For the purposes of this study, to provide quick image visibility improvement, the Contrast Limited Adaptive Histogram Equalization (CLAHE) method was selected (Fig. 1A). This provides better visibility of ichnological features in a short time through the local contrast of an image based on modification of two main parameters: i) block size, controlling the size of the local region around a pixel for which the histogram is equalized; and ii) histogram bins, defining the number of bins used for histogram equalization, which should be smaller than the number of pixels in a block.

For the quantification of bioturbated surface, after applying CLAHE, the obtained image must be converted to an 8-bit grayscale image. Then, the threshold has to be defined to establish a differentiation range for black and white by means of the *Image* menu. The determination of this parameter is supported by a preview screen, where the potential image is observed, and the value can be modified to obtain a more appropriate bioturbated differentiation (black pixels were assigned to bioturbation). The enhanced black and white binary image can be used to quantify the amount of bioturbated surface (Fig. 1B left). The Fiji process menu offers a wide range of easy-to-use binary tools and filters (e.g., dilate, erode, fill holes and skeletonize, among others). After testing, the following ones were selected as the most suitable for ichnological analysis: a) Erode: it removes erratic black pixels from the edges of bioturbation, proving extremely useful to delete minor compounds of black pixels that do not correspond to trace fossils, and thereby more precisely discern the shape of the burrows; b) Fill holes: it fills white pixels located inside the trace fossils that are surrounded by black ones; and c) Minimum/Maximum filters: they perform binary erosion by replacing each pixel in the image with the smallest/largest pixel value in that pixel's neighborhood, helping one to remove small black pixels that conform background noise (Fig. 1B right). All these tools and filters present a preview option, which makes it easy, quick and intuitive to select the size of the neighborhood.

Finally, manual corrections can be conducted using eraser or painting tools, controlling that all the selected pixels belong to trace fossils. Afterwards, output measurement results are quickly obtained with the Fiji analyze menu, whose program measures the percentage of black pixels, hence offering a quantification of the bioturbated surface.

2.2. ICY

ICY software uses an adaptive histogram equalization to enhance trace fossil visibility (Fig. 2A). This plugin controls parameters similar to Fiji´s, but through a distinctive automatic procedure, giving different results.

When it is used to quantify bioturbated surface, ICY software can extract part of the image according to pixel values via a thresholding menu. The pixel selection can be done through manual adjustment, where threshold values are defined to derive a binary image (Fig. 2B left). The selection may also be obtained automatically using the K-Means tool, whose algorithm calculates the threshold value after defining the number of "classes" (i.e., areas in the histogram) to be differentiated (value 2 for binary images). In the case of abundant background noise, a high threshold value should be chosen to ensure that bioturbation is well delimited and assigned to white pixels (Fig. 2B left). The derived image can then be exported and inverted by Fiji. A final filtering treatment and estimation of bioturbated surface should be conducted using Fiji, repeating the process, to arrive at improved yet rapid results (Fig. 2B right).

Finally, the Intensity Profile plugin was used here to characterize the penetration depth of trace fossils with passive filling (i.e., material that filled a burrow by physical sedimentation after its occupant departed; Bromley, 1990). This plugin calculates the intensity value of each pixel (obtaining three values per pixel, one for each channel) along a particular Region Of Interest (ROI). The values are plotted on intensity profile graphics from a given ROI (vertical line along the core), after which they must be manually checked and filtered to identify potential mistakes. The final graphs of intensity values along the ROI are used to compare intensity values from the infilling material with those from the host sediment. Similar values allow one to recognize the colonization horizon and then estimate the penetration depth of passively filled trace fossils from this horizon.

2.3. High-resolution image treatment

The tested high-resolution image treatment for enhancing trace fossil visualization is based on the method defined by Dorador et al. (2014). This technique modifies some image adjustments (i.e., *levels*, *brightness* and *vibrance*) to increase the contrast between the host sediment and the infilling material. It was initially proposed in sediment core research, and later it was tested in rock samples after minor modification (see Dorador and Rodríguez-Tovar, 2018, for a review).

To characterize the percentage of bioturbated surface, Dorador et al. (2014b) presented a package consisting of three selection tools: Similar Pixel Selection Method (SPSM), Magic Wand Method (MWM) and Color Range Selection Method (CRSM). In the present study, to further enhance quantification, selections obtained from each of the above methods (Fig. 3B left) were slightly modified using a novel resource called RefineEdge (Fig. 3B right). It enables one to refine the selected area, corresponding to the biogenic structure, by modifying five parameters (*Radius*, *Smooth*, *Feather*, *Contrast* and *Shift Edge*), giving rise to a better characterization of the discrete trace fossil. The *Radius* parameter controls the size of the border in which refinement is applied. Once the size is defined, *Smooth*, *Feather* and *Contrast* are modified. *Smooth* makes it possible to remove hills and valleys, smoothing the border; *Feather* smudges the boundary between the selected area and surrounding pixels; and *Contrast* modifies transitions along the border. Finally, borders can be moved inward or outward by means of the *Shift Edge* parameter, controlling the final selected area.

Penetration depth is determined by high-resolution digital image treatment following quantitative pixel analysis, as was proposed by Dorador and Rodríguez-Tovar (2014). Host sediment pixels and those from the infilling material of trace fossils are quantified and plotted, and the comparison between pixel values allows the penetration depth to be evaluated (Dorador and Rodríguez-Tovar, 2014).

2.4. Data set

Numerous images from sediment cores and outcrops were considered in this study. For core samples, the data set consisted of high-resolution images from IODP Expedition 339, subjected to numerous ichnological studies during recent years (e.g., Rodríguez-Tovar and Dorador, 2014; Rodríguez-Tovar et al., 2015a, b; Dorador and Rodríguez-Tovar, 2016a, 2018). Core images were from 1.5 m sections split lengthwise and scanned (on the Section Half Imaging Logger) on board during the cruise (Expedition 339 Scientists, 2013a). Fifty-four of these core samples were selected, and 10-20 cm core intervals were cropped from every core section in view of potentially interesting ichnological content. Additionally, two core segments were selected from IODP Site U1385 corresponding to core depths of 40.05 to 40.20 meters (U1385A-5H-CC) and 70.7 to 70.9 meters (U1385-8H-5A) to analyze the penetration depth. They are mainly hemipelagic claystones, with no primary sedimentary structures, but many discrete trace fossils over a mottled background (Expedition 339 Scientists, 2013b, Rodríguez-Tovar and Dorador, 2014).

Several images of outcrop samples from the Cyprus carbonate contourite drift succession (Petra Tou Rominou section; see Stow et al., 2002) and Moroccan Miocene sandy contourite channels (Capella et al., 2017) were furthermore considered. Ichnological studies of these rocks reveal informative examples of variable relationships between trace fossils and host sediment. Before taking photographs (Canon ® PowerShot SX420), outcrops were sprayed with water to improve trace fossil visibility. More than 100 images corresponding to several lithologies (chalk, calcilutites, calcarenites) were treated to evaluate different cases (for chalk, see Miguez-Salas and Rodríguez-Tovar, 2019). Finally, based on the contrast between the trace fossils and the surrounded material, three images were selected as illustrative and representative examples: calcarenite (high contrast), chalk (medium contrast) and calcilutite (low contrast).

3. Comparative analysis

Comparison of the selected methods (i.e., Fiji, ICY and high-resolution image treatment) served to highlight the strengths and weaknesses of each in improving the visibility of trace fossils and quantification of bioturbated surface. In the case of high-resolution image treatment and ICY, we were able to compare the penetration depth as well.

3.1. Enhancing visibility of bioturbation

The techniques were applied on outcrop images to increase the visibility of trace fossils, and consequently facilitate ichnotaxonomical identification. For a detailed comparison, three examples were selected based on the original contrast (high, medium and low contrast) between trace fossils and the surrounding sediment (Fig. 4).

Application of the high-resolution image treatment served to enhance the visualization of trace fossils in the three examples, being particularly successful in low and medium contrast examples, where some ichnotaxa could not be identified in the original image. Reliable identification and characterization of some *Chondrites* was only possible after the treatment (Fig. 4).

The CLAHE methodology conducted with Fiji provides good results for medium to low contrast samples, especially for highlighting *Thalassinoides* and *Planolites* (Fig. 4). However, differentiation of *Chondrites* is still difficult after treatment. In the case of the high contrast example, visualization of traces such as *Chondrites* is aided by treatment (Fig. 4).

Adaptive histogram equalization in ICY gives excellent results for the reddish *Chondrites* of the high-contrast image (Fig. 4), although improvement in trace fossil visualization is relatively low in those images with medium to low contrast between trace fossils and surrounding sediment (Fig. 4).

3.2. Quantification of bioturbated surface corresponding to discrete trace fossils

High-resolution image treatment entailed using the refined versions of Color Range Selection Method (CRSM), Magic Wand Method (MWM) and Similar Pixels Selection Method (SPSM) on high, medium and low contrast examples (Fig. 5). The three techniques provided similar values for bioturbated surface corresponding to discrete trace fossils in the high contrast example —from 9% to 12%— indicating a low degree of bioturbation (BI = 2, according to bioturbation index of Taylor and Goldring, 1993). These differences are higher in the medium contrast image, where quantification ranges from 10% to 19%, although both values correspond to the same bioturbation index (BI = 2). Finally, application on the low contrast example gives more variable values, from 19% to 47%; estimations obtained with CRSM and MWM represent a low index of bioturbation (BI = 2), while that from SPSM corresponds to a higher index (BI = 3).

In conjunction with Fiji and ICY methods, the amount of bioturbated surface corresponding to discrete trace fossils was calculated before and after filtering binary images (Figs. 1, 2 and 5). Both techniques show similar results in the high contrast outcrop image (15% and 16%) pertaining to a low bioturbation index (BI = 2). The results obtained from medium (16% and 20%) and low (19% to 22%) contrast images are also similar, corresponding, at any rate, to a low bioturbation index (BI = 2 for all). Filtering was less complex in the case of binary images obtained with Fiji methodology. In the case of ICY method, the filtering was especially difficult due to the assignment of some host sediment areas as pixels belonging to trace fossils.

3.3. Penetration depth

Penetration depth values were calculated, and then compared, using both the quantitative pixel analysis proposed by Dorador and Rodríguez-Tovar (2014), and the Intensity Profile plugin by ICY (IP-ICY). In both cases, images were previously treated with high-resolution image treatment and Fiji (CLAHE), respectively, to improve trace fossil visualization. As explained previously, we focus on passively filled trace fossils; comparison between data (mean pixel values in case of the high-resolution image treatment and intensity values in case of IP-ICY) from the infilling material with those of the host sediment enabled us to approach the colonization horizon and then estimate the penetration depth. Two core segments from IODP Site U1385 having different contrast between the fill material of trace fossils and the surrounding sediments were studied: a higher contrast image corresponding to U1385A-5H-CC, and a lower contrast image to U1385A-8H-5A (Fig. 6).

In general, the results of the two methods were similar, but some noteworthy differences should be pointed out (Fig. 6). For the higher contrast example, the high-resolution image treatment improved visualization of three *Thalassinoides* cross-sections and the quantitative pixel analysis allowed the identification of corresponding penetration depths of 2.8, 3.6 and 9.8 cm (Fig. 6A left). The IP-ICY shows two intensity value packages that can be linked to *Thalassinoides*; the first records penetration depths of 2.6, 3.5 and 5.5 cm, and the second shows lower intensity values, giving an estimated penetration depth of 9.5 cm (Fig. 6A right). For the low contrast core interval, according to quantitative pixel analysis, four *Thalassinoides* cross-sections showed penetration depths of 1.8, 6.3 and 10.0 (in two cases) cm (Fig. 6B left). Using IP-ICY it is more difficult to distinguish between the fill of trace fossils and the host sediment, although four different intensity value packages might be associated with the four *Thalassinoides* cross-sections, having penetration depths of 2, 9, 10 and 11 cm.

4. Discussion

This study confirms that image processing techniques facilitate trace fossil analysis when applied to characterize particular ichnological features such as ichnodiversity, quantification of bioturbation, and penetration depth. Our comparative analysis furthermore served to evidence strengths and weaknesses of the three studied methodologies (Table 1).

In terms of enhanced trace fossil visualization —facilitating trace fossil differentiation and therefore ichnotaxonomical classification— the three methods provide good results for high contrast images (Fig. 4). Results for mid and low contrast examples show some differences depending on the applied method, with better results after applying high-resolution image treatment and Fiji (CLAHE), and weaker visualization after adaptive histogram by ICY. Given the rapid processing of Fiji, this option may be seen as an alternative to the time-consuming high-resolution image treatment. This approach is especially promising for preliminary analyses, since the outstanding results on cores (Fig. 6) may be helpful when selecting the most appropriate core segments during the initial stages of a research study. In sum, the Fiji quick treatment facilitates decision-making in studies involving a great number of cores or outcrop samples. More detailed analysis can be conducted later through the application of high-resolution image treatment. Furthermore, whereas Fiji and ICY work with black and white binary images, the fact that high-resolution image treatment allows one to apply different colors according to the pixel values makes ichnotaxonomical classification easier.

All the applied methods quantify the abundance of bioturbation exclusively to discrete trace fossils (having differentiated outlines and characteristic shapes), without any evaluation of the biodeformational structures or bioturbated texture (undifferentiated outlines with no definitive geometry, a mottled background). However, after characterization of the presence/absence of a mottled background, quantification of the bioturbated surface corresponding to discrete trace fossils is indeed of interest, bearing a relationship with palaeoenviromental conditions.

The overall comparison of the different methodologies tested indicates that all of them provide good results for high contrast examples, ranging from 9% to 16% of bioturbated surface (Fig. 5), which belong to a low degree of bioturbation (BI = 2) (Fig. 5). Results are similar for medium contrast images (10%–20%), belonging to the same bioturbation index (BI = 2). At the opposite end, application on low contrast images shows very substantial differences, from 19% to 47%, though most values are around

20% (the exception being SPSM + RefineEdge). Accordingly, and taking into account that CRSM underestimates the amount of bioturbation, as some trace fossil pixels are not selected, the most successful selection tool would be Magic Wand Method (+ RefineEdge), providing very realistic results in all the studied examples. Since MWM is time-consuming, especially when working with small trace fossils (Dorador et al., 2014b), Fiji stands as an attractive alternative for low contrast samples, and ICY (+ Fiji) for medium contrast ones. For precise results both programs (Fiji and ICY) require complex filtering; however, the results of initial automatic estimation without filters are not very different from the final values (Figs. 1 and 2), meaning they lend themselves to rapid initial appraisals. Finally, in high contrast images, any method can be applied —all three offer realistic results about the bioturbated surface corresponding to discrete trace fossils. Again, an important advantage of any high-resolution image treatment over Fiji and ICY resides in the possibility to estimate the percentage of area occupied for each ichnotaxon, the whole icnocoenosis, or the complete ichnofabric (Rodríguez-Tovar and Dorador, 2015). Fiji and ICY only allow for quantification of the whole bioturbated surface as they work with binary images.

In terms of penetration depth, good results were obtained for the core examples after either method, quantitative pixel analysis in the case of high-resolution image treatment or the intensity values in the case of IP-ICY. Still, the most appropriate procedure might be selected in light of the particular contrast of the images at hand. When dealing with a higher contrast image, both methods show similar values for penetration, supporting the obtained values; but the record of two intensity value packages with IP-ICY might indicate two different communities of *Thalassinoides* tracemaker, which could have paleoecological implications. For the low contrast core section, the scarce differentiation between the values associated with the fill of trace fossils and those from the host sediment in the IP-ICY analyses would impede a precise estimation, which can be obtained with the quantitative pixel analysis. Nonetheless, as in the previous case, data from IP-ICY allow discernment between two different communities of *Thalassinoides* tracemaker coming from two distinct horizons of colonization. Although both methods are valuable for estimating penetration depth, the semi-automatic character of IP-ICY favors faster calculation. In short, IP-ICY is a promising tool for characterizing the penetration depth of some trace fossils in a very rapid and intuitive way, especially for high contrast images, facilitating even the differentiation between distinct horizons of colonization. In any case, this approach must be supported by a precise ichnological analysis to avoid misinterpretation of the obtained pixel values, as those determined by reworking of biogenic structures (e.g., *Thalassinoides* reworked by other traces as *Chondrites*).

4.1. The applied techniques: consequences for paleoenvironmental research

Conducted research supports the usefulness of the image processing techniques evaluated here when characterizing particular ichnological features: ichnodiversity, quantification of bioturbation, and penetration depth. All these features are of major interest in any ichnological study, and especially under the ichnofabric approach (McIlroy, 2004; Buatois and Mángano, 2011; Ekdale et al., 2012) and the ichnofacies model (Buatois and Mángano, 2011; MacEachern et al., 2012). Both these paradigms in ichnological analysis have become consolidated as significant proxies to interpret sedimentary environments (Knaust and Bromley, 2012), based on the characterization of ecological and depositional conditions.

The ichnofabric approach has been successfully used as a tool in a range of Earth Science disciplines, but particularly for paleoenvironmental research and sedimentary basin analysis, and recently for reservoir characterization (McIlroy, 2004; Buatois and Mángano, 2011; Ekdale et al., 2012). Detailed ichnofabric analysis calls for integrating ichnofabric attributes, including ichnotaxa diversity, ichnological features (i.e., dimensions of ichnotaxa), amount of bioturbation, cross-cutting relationships, and/or tiering structures (Taylor et al., 2003). Such ichnofabric attributes are controlled by depositional and ecological conditions: grain size, nature of substrate, oxygenation, nutrient levels, salinity, and sedimentation rate, among others, determined by particular depositional processes (Taylor et al., 2003). On this basis, the applied image processing techniques would facilitate ichnofabric characterization, hence environmental interpretations.

Because the ichnofacies model looks into groups of trace fossils that reflect animal responses (ethology) to paleoenvironmental conditions (Buatois and Mángano, 2011; MacEachern et al., 2012), a detailed ichnofacies characterization proves essential for paleoenvironmental analysis and sedimentary basin research. Means of improving ichnotaxonomic analysis is important not only for the recognition and differentiation of ichnofacies; it becomes fundamental when working with cores from modern marine sediments showing a comparatively weak differentiation of discrete trace fossils.

Therefore, we strive to demonstrate the utility of the applied methods in the case of modern marine sediments that are mainly retrieved from deep-marine settings (Dorador et al., 2014a, b; Rodríguez-Tovar and Dorador, 2015) and ichnofacies (Dorador and Rodríguez-Tovar, 2015). Depositional processes (i.e., pelagic, gravitational and bottom-current) determining variations in environmental conditions (i.e., organic matter deposition, oxygenation of the pore water, etc.) can be approached through deep-sea ichnological research (Wetzel, 2010; Uchman and Wetzel, 2011; Wetzel and Uchman, 2012), and facilitated by novel applications.

Image processing techniques should therefore be considered as an essential tool for sedimentary basin research, supported by ichnological information, and not only when working with marine cores from modern deposits, but also based on outcrop studies.

5. Conclusions

The usefulness of Fiji and ICY methods for ichnological studies has been tested and compared with high-resolution image treatment. Concretely, their efficiency in the enhancing of trace fossils visibility, quantification of the bioturbation and estimation of penetration depth were tested.

High-resolution image treatment (+ RefineEdge) is revealed as the best option to increase visibility of trace fossils. However, Fiji (CLAHE) proved to be a rapid alternative for preliminary analysis of cores when selecting the most appropriate segments in the initial stages of research.

Regarding the quantification of the bioturbation, the Magic Wand Method (+ RefineEdge) provides good results regardless of image contrast, allowing for quantification of the area occupied by each ichnotaxon, the whole icnocoenosis, or the complete ichnofabric. Notwithstanding, Fiji and ICY (+ Fiji) stand as faster alternatives to quantify the whole of the bioturbated surface, especially recommended in early research stages.

Quantitative pixel analysis and IP-ICY are useful for estimating penetration depth, but the semi-automatic character of IP-ICY favors speedy calculation.

Therefore, use of the newly tested techniques (Fiji and ICY) is highly recommended during early stages of research as an initial approach or to filter the adequate representative samples. The great potential of these techniques should be further explored in the realms of ichnological analysis, and particularly for sedimentary basin research, as ichnological information reported thus far is well supported and quite promising.

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FIGURE CAPTIONS

Fig. 1. Example of Fiji treatment stages to enhance trace fossil visualization (A) and quantify the bioturbated surface (B). Scale bars = 1cm.

Fig. 2. Example of ICY treatment stages to enhance trace fossil visualization (A) and quantify the bioturbated surface (B). Note that bioturbation is assigned to white pixels and the last stages of filtering and inversion are assisted by Fiji. Scale bars = 1cm.

Fig. 3. Example of high-resolution image treatment application to enhance trace fossil visibility by the modification of image adjustments (A) and for quantification of bioturbated surface (B). SPSM, Similar Pixel Selection Method; MWM, Magic Wand Method; CRSM, Color Range Selection Method. Scale bars = 1cm.

Fig. 4. Original images and obtained results after application of high-resolution image treatment, CLAHE extension by Fiji, and the adaptive histogram by ICY on high, medium

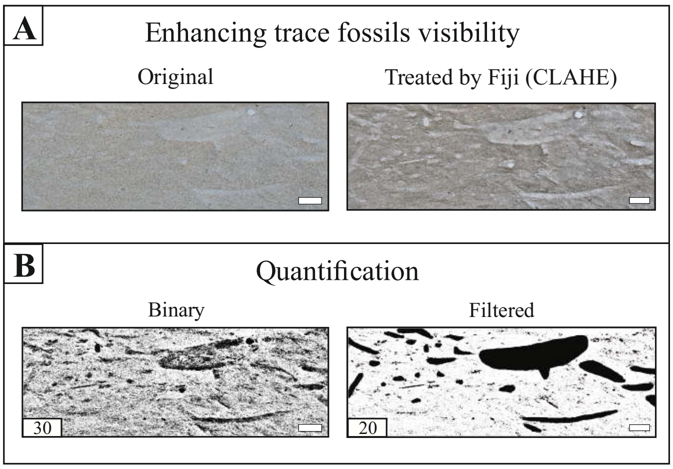
and low contrast samples. Scale bars = 1cm. *Pl*, *Planolites*; *Th*, *Thalassinoides*; *Ch*, *Chondrites*.

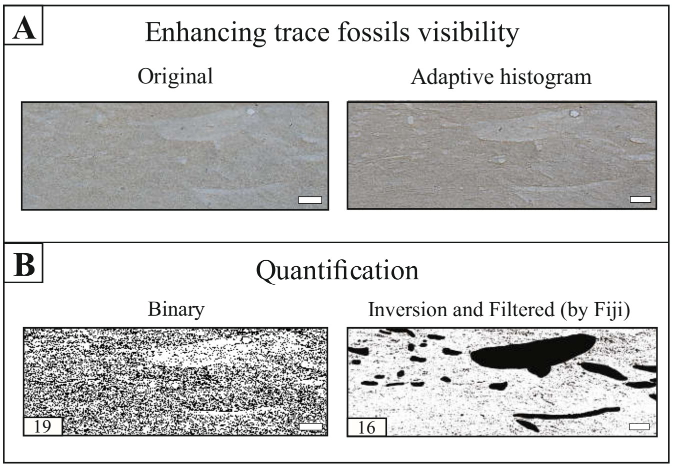
Fig. 5. Quantification of percentage of bioturbated surface by different methods of high-resolution image treatment, Fiji and ICY software. CRSM, Color Range Selection Method; MWM, Magic Wand Method; SPSM, Similar Pixel Selection Method. Scale bars = 1cm.

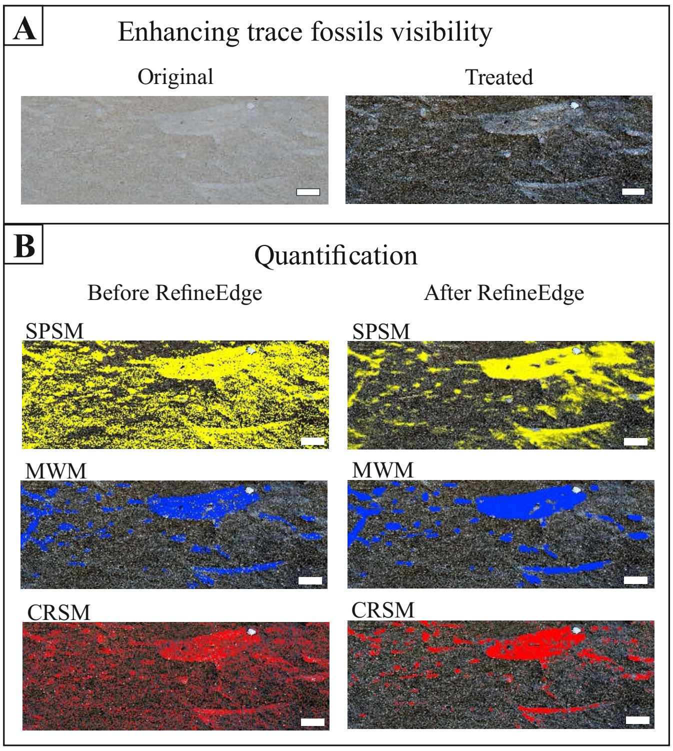
Fig. 6. Penetration depth of trace fossils obtained with quantitative pixel analysis (left) and IP-ICY (right) methodology in high (A) and low (B) contrast core examples. Note: left scale in cm.

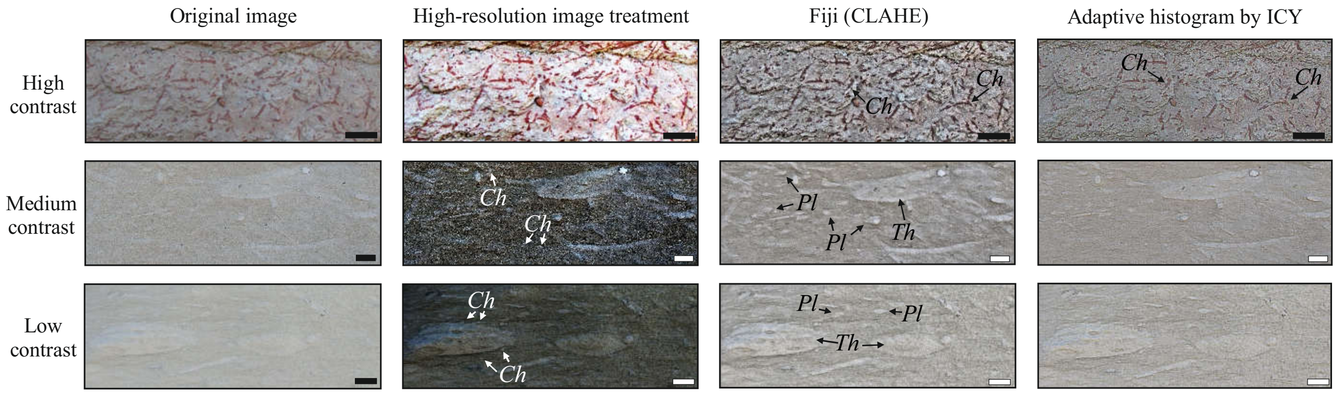
TABLE CAPTION

Table 1. Summary of strengths and weaknesses of each methodology depending on the purpose.

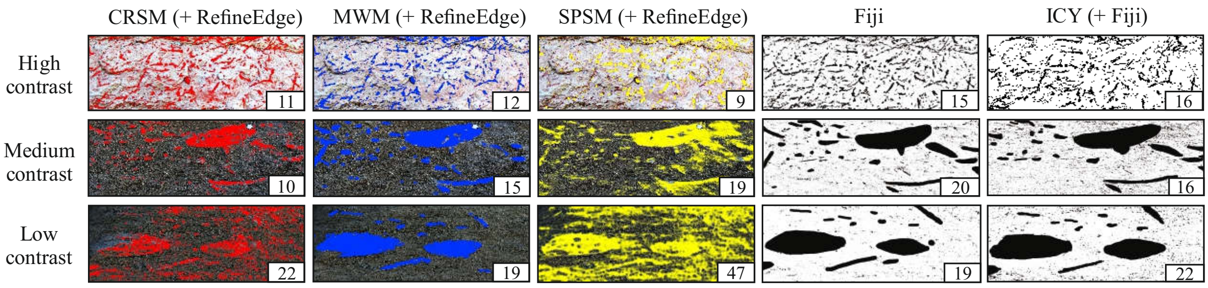

A
Enhancing trace fossils visibility
Original
Treated by Fiji (CLAHE)
B
Quantification
Binary
Filtered
30
20


A
Enhancing trace fossils visibility
Original
Adaptive histogram
B
Quantification
Binary
Inversion and Filtered (by Fiji)
19
16

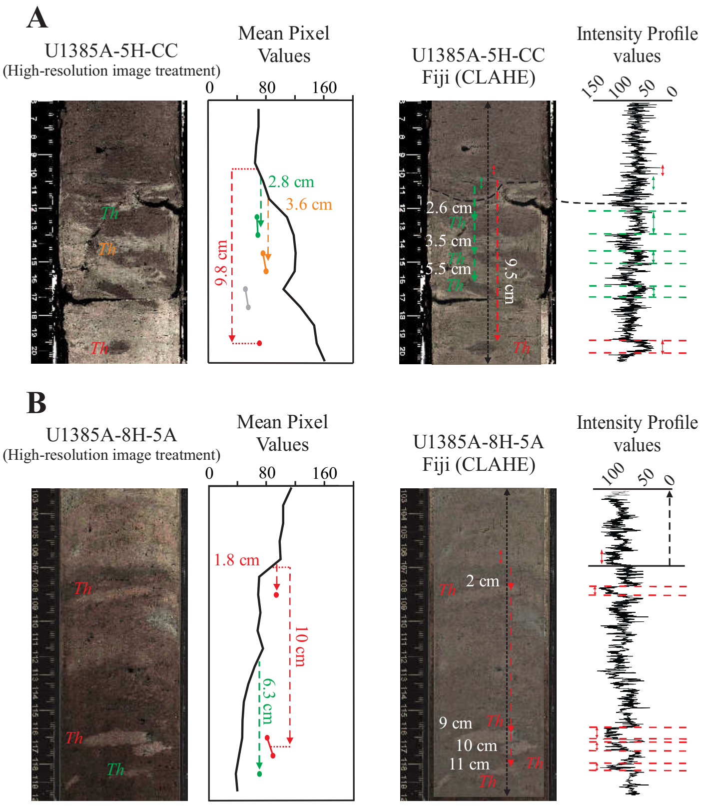

A
Enhancing trace fossils visibility
Original
Treated
B
Quantification
Before RefineEdge
After RefineEdge
SPSM
SPSM
MWM
MWM
CRSM
CRSM



Original image
High-resolution image treatment
Fiji (CLAHE)
Adaptive histogram by ICY
High contrast
Medium contrast
Low contrast
Ch
Ch
Ch
Ch
Ch
Ch
Ch
Ch
Pl
Pl
Th
Pl
Pl
Th



CRSM (+ RefineEdge)
MWM (+ RefineEdge)
SPSM (+ RefineEdge)
Fiji
ICY (+ Fiji)
High contrast
Medium contrast
Low contrast
11
12
9
15
16
10
15
19
20
16
22
19
47
19
22


A
U1385A-5H-CC
(High-resolution image treatment)
Mean Pixel Values
0
80
160
Th
Th
Th
2.8 cm
3.6 cm
9.8 cm
U1385A-5H-CC Fiji (CLAHE)
2.6 cm
3.5 cm
5.5 cm
9.5 cm
Intensity Profile values
150
100
50
0
B
U1385A-8H-5A
(High-resolution image treatment)
Mean Pixel Values
1.8 cm
10 cm
6.3 cm
U1385A-8H-5A Fiji (CLAHE)
2 cm
9 cm
10 cm
11 cm
Intensity Profile values
100
50
0

Methods	Enhancing visibility of trace fossils	Quantification of bioturbated surface	Estimation of penetration depth
High-resolution image treatment (+ RefineEdge)	Good results for any image (high to low contrast), based on the modification of three image adjustments (*levels*, *brightness* and *vibrance*). Application of different colors facilitates ichnotaxonomy.	CRSM and MWM provide reasonable values for high and medium contrast images. MWM is also useful in low contrast images, but it is time-consuming. Application of different colors allows detailed quantification.	Good estimation, but time-consuming.
Fiji	It provides good results for any image in a rapid way. Binary character makesichnotaxonomical differentiation difficult.	Good for high and low contrast images, but after complex filtering. Only for quantification of total bioturbated surface.	Not possible.
ICY	Successful for high contrast images. Binary character makes ichnotaxonomical differentiation difficult.	Good for high and medium contrast images, but it requires complex filtering. Note that the last stages have to be conducted with Fiji. Only for quantification of total bioturbated surface.	Good estimation in a very rapid way. Facilitates differentiation between specimens.