

Recognizing the heritage behind Hugo Díaz's caricature for the Costa Rican children's novel Cocorí using non-destructive techniques

Johnny Buzo-Chacón

Universidad de Costa Rica

Roberto Montero-De la Paz

Universidad de Costa Rica

Laura Raabe-Cercone

Universidad de Costa Rica

Daniela Jaikel-Viquez

Universidad de Costa Rica

Alejandra Gómez-Arrieta

Universidad de Costa Rica

Stefany Lozada-Alvarado

Universidad de Costa Rica

Alejandra Rojas-González

Universidad de Costa Rica

Mario Cubero-Campos

Universidad de Costa Rica

Mauricio Redondo-Solano

Universidad de Costa Rica

Óscar Andrey Herrera-Sancho

`oscar.herrerasancho@ucr.ac.cr`

Universidad de Costa Rica

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1 Recognizing the heritage behind Hugo Díaz's 2 caricature for the Costa Rican children's novel *Cocorí* 3 using non-destructive techniques

4 J.G. Buzo-Chacón¹, R. Montero-De la Paz¹, L. Raabe-Cercone^{2,3}, D. Jaikel-Viquez^{4,5,6}, A.
5 Gómez-Arrieta⁴, S. Lozada-Alvarado⁷, A. M. Rojas-González⁸, M. Cubero-Campos^{1,9}, M.
6 Redondo-Solano^{4,6,10}, and O.A. Herrera-Sancho^{1,5,9,11,*}

7 ¹Escuela de Física, Universidad de Costa Rica, 2060 San Pedro, San José, Costa Rica

8 ²Museo de la Universidad de Costa Rica, 2060 San Pedro, San José, Costa Rica

9 ³Centro de Investigación en Identidad y Cultura Latinoamericanas, 2060 San Pedro, San José, Costa Rica

10 ⁴Facultad de Microbiología, Universidad de Costa Rica, 2060 San Pedro, San José, Costa Rica

11 ⁵Instituto de Investigaciones en Arte, Universidad de Costa Rica, 2060 San Pedro, San José, Costa Rica

12 ⁶Centro de Investigación en Enfermedades Tropicales, Facultad de Microbiología, Universidad de Costa Rica, 2060
13 San Pedro, San José, Costa Rica

14 ⁷Laboratorio Clínico y Banco de Sangre de la Universidad de Costa Rica, Universidad de Costa Rica, 2060 San
15 Pedro, San José, Costa Rica

16 ⁸Escuela de Ingeniería de Biosistemas, Universidad de Costa Rica, 2060 San Pedro, San José, Costa Rica

17 ⁹Centro de Investigación en Ciencias Atómicas Nucleares y Moleculares, Universidad de Costa Rica, 2060 San
18 Pedro, San José, Costa Rica

19 ¹⁰Laboratorio de Investigación y Entrenamiento en Microbiología de Alimentos y Aguas, Universidad de Costa Rica,
20 2060 San Pedro, San José, Costa Rica

21 ¹¹5. Physikalisches Institut, Universität Stuttgart, Pfaffenwaldring 57, 70569, Stuttgart, Germany

22 *oscar.herrerasancho@ucr.ac.cr

23 ABSTRACT

Caricatures are generally small-format artworks typically published in the press with a limited lifespan, because they are designed to help shape public opinion for brief consumption. Additionally, original illustrations for editorial purposes rarely endure over time. We employed non-destructive techniques, approaches used in other types of artworks, e.g., oil paintings, in order to establish a baseline for the conservation state of cartoonist Hugo Díaz's illustration for the famous Costa Rican children's novel *Cocorí* by Joaquín Gutiérrez. We focused on identifying priority attention zones within the caricature to create a visible and understandable map that would facilitate the location of future findings. Using non-destructive visible-light microscopy, we quantitatively assessed the paper's grammage and examined how various degradation mechanisms—such as mechanical stress, chemical oxidation, or material displacement—affected the density and distribution of the paper matrix. This approach allowed us to precisely determine the zones most likely to harbor damage and microorganisms, leading to the isolation and identification of a bacterial and a fungal strain. We also identified the caricature's colour palette, which includes Burnt Sienna or Umber, Ultramarine Blue, and Naples Yellow or Raw Sienna.

25 1 Introduction

26 Artwork surrounds us in many forms, embodying specific societal values shaped by the practices, ideologies, and beliefs
27 of individuals and collectives; furthermore, it is the result of technological advances and scientific discoveries. Thus, an oil
28 painting—whose value lies in its originality and enduring nature—is not the same as an image created for technical reproduction,
29 such as an illustration for a book or newspaper. Caricature emerges when an artist chooses to infuse an artwork with exaggerated,
30 distorted, humorous, critical, or irreverent elements—or expressions of opinion—designed to make an impact within a specific
31 socio-political context, without necessarily aiming for posterity. The advancement of caricature and book illustration is closely
32 linked to the development of the press, the formation of public opinion, and improvements in printing techniques. Consequently,
33 these artworks are typically small-scale pieces created for reproduction via printing processes; as such, they are often made
34 from materials with limited durability, since reproducibility is prioritized, meaning that a short lifespan is frequently inherent
35 to the very nature of the caricature. The following study presents the results of a multidisciplinary investigation conducted

on a watercolour painting made by Costa Rican artist and cartoonist Hugo Díaz Jiménez (1930-2001), in 1995. This iconic artwork illustrates the cover of the 1995's version of the Costa Rican children's novel *Cocorí* (see Figure 1); written by Joaquín Gutiérrez Mangel (1918-2000) (published by the Central American University Press [known by its Spanish acronym, EDUCA]).

Caricature is a graphic art that combines iconic and linguistic codes. It is a versatile type of visual creation, as it can employ a variety of compositional resources and different artistic techniques¹. The word caricature comes from the Italian word *caricare*, which means to load or exaggerate; that is, it designates a satirical drawing that usually exaggerates a character's features with a humorous and satirical purpose. This term was adopted in other languages, such as Spanish (*caricatura*), French (*caricature*), and German (*karikatur*). In English, the term cartoon comes from the Italian word *cartone*, which is used to designate a strong and heavy paper, or to refer to preliminary sketches made on that type of paper. Since the 19th century, this word has been used to designate satirical drawings published in magazines and newspapers². Although drawings with humorous or satirical intent can be identified since antiquity, caricature is a modern artistic production. During the Italian Renaissance, renowned artists—such as Holbein, Da Vinci, and Dürer—created satirical drawings. However, the Carracci brothers are considered the pioneers of caricature in late 16th-century Bologna². During the 18th century, caricature and satirical drawing experienced tremendous growth due to the influence of Enlightenment ideas and the rise of critical thinking. Furthermore, improvements in engraving techniques contributed to its dissemination. Later, during the 19th century, caricature experienced a significant expansion, thanks to advances in the mechanization of image reproduction and technical improvements in printing. As a result, satirical newspapers incorporating caricatures emerged in Europe and the United States of America³. In Latin America, caricature also saw significant development due to the expansion of the press and print culture following the independence movements of the 19th century.

In the specific case of Costa Rica, Hugo Díaz Jiménez was one of the most renowned cartoonist and illustrators. Since his childhood days, he demonstrated a remarkable talent for drawing and received guidance from his uncle-in-law, the caricaturist Noé Solano Vargas (1899-1971). As a student at the Lyceum of Costa Rica, he studied drawing under Francisco Amighetti Ruiz (1907-1998) and Juan Manuel Sánchez Barrantes (1907-1990), both prominent artists of the 1930s generation of the history of art in Costa Rica⁴. In 1944, Díaz published his first caricatures in *La Hora* and the magazine *Vértice*, published by students of the Lyceum of Costa Rica. It is important to note that during the 1930s, Costa Rica experienced a boom in caricature due to the expansion of the press and the growth of the artistic and cultural scene^{4,5}. In contrast, during the 1950s and 1960s, cartooning declined, as the local press opted to publish foreign comic strips, mainly from the United States of America; due to the high cost of producing printing plates for cartoons. Thus, during his youth, Hugo Díaz was unable to pursue a professional career in caricature, because the newspapers were not interested in buying his work. During those years, Díaz worked as a cartographic draftsman, engineering draftsman, and advertising designer⁶.

Beginning in the 1970s, Costa Rican cartooning experienced a resurgence. On one hand, the press became interested in publishing cartoons produced by local artists, thanks to the adoption of technical improvements that reduced printing costs⁴. On the other hand, since the 1960s, the Costa Rican government had promoted its main cultural policies of patronage and dissemination: Editorial Costa Rica (1959), the National Culture Awards (1962), and the General Directorate of Arts and Letters (1963). In 1971, the Ministry of Culture, Youth, and Sports of Costa Rica was founded, further expanding these policies. Consequently, the visual arts experienced remarkable development, thanks to the creation of various exhibition spaces, such as the Halls of Visual Arts (1972-1993), the Enrique Echandi Exhibition Hall (1972), and the Museum of Costa Rican Art (1977). In addition, specific spaces for the dissemination of caricatures were promoted, such as exhibitions and competitions. These initiatives were instrumental for the growth of the Costa Rican cultural field⁷.

It is worth noting that Hugo Díaz distinguished himself as a book illustrator. Although he had undertaken illustration projects for advertising since the 1960s, it was not until the 1970s that illustration commissions became regular. In this regard, the founding of Editorial Costa Rica, university presses, and private publishing houses was crucial. Díaz's professional association with EDUCA, part of the Central American Higher University Council (known by its Spanish acronym, CSUCA), was particularly significant; he worked on projects for them from 1975 until 1999, when CSUCA relocated to Guatemala. For this publishing house, Díaz created illustrations for important works of Costa Rican and Central American children's literature, such as: *Los cuentos de mi Tía Panchita* by Carmen Lyra (EDUCA, 1982), *Cocorí* by Joaquín Gutiérrez Mangel (EDUCA, 1983), *Tío Coyote y Tío Conejo* by Nicaraguan writer Pablo Antonio Cuadra (EDUCA, 1998), *El conejito* by Panamanian writer Rogelio Sinán (EDUCA, 1998), and *La cucarachita Mandinga* by Carmen Lyra (EDUCA, 1998)^{8,9}. All these children's illustration projects share a similar style: ink outlines, watercolour figures with transparent and bright colours, rounded shapes that emphasize the characters' tenderness, and an anthropomorphic representation of the animal characters.

Throughout his career, Díaz received numerous accolades for his artwork: The Award for Best Humorous Cartoon at the First Annual Noé Solano Cartoon Hall (Costa Rica, 1970), The 2nd Prize in Editorial Cartoon at the Graphic Humor Pavilion in Montreal (Canada, 1972), the Joaquín García Monge National Journalism Award (Costa Rica, 1976), the Aquileo J. Echeverría Award in Plastic Arts (Costa Rica, 1995) and the Pío Víquez National Journalism Award (Costa Rica, 2000)⁹.

As previously stated, in 1983, Díaz created the illustrations for the novel *Cocorí*, first published in 1947 (Editorial Rapa

Nui, Chile). His artwork gave the novel's characters a distinct visual identity. The versions of *Cocorí* illustrated by Díaz had 12 reissues, from 1983 to 1999. In 1995, Díaz had to redo the illustrations because EDUCA changed the book's format from landscape to portrait⁷. The cover of this specific edition is the artwork that is going to be analyzed in the present study.

Cocorí is the story of a young Afro-Caribbean boy, aged seven, who lives in Limón, Costa Rica, with his mother Drusila. As the story unravels, he embarks on a journey into an unknown tropical jungle after receiving a rose as a gift from a little girl who arrives by ship. The beauty and fragility of the rose became a source of deep insightfulness for *Cocorí* when, shortly after, the flower died. This event triggered in him a state of reflection and multiple unknowns, which generated a drastic change in his life and constituted the main reason for undertaking his journey¹⁰. All the natural elements described in the book, the vegetation and the fauna majestically come to life in Díaz's paintings. His illustrations are part of the visual identity of this emblematic work, contributing to generations of readers imagining the characters and settings, thus, becoming a referent of Costa Rican illustration. For several decades, *Cocorí* was in high demand, as it was mandatory reading in the Ministry of Public Education's school programs. However, in 2003 it became optional due to a controversy regarding its racial depictions.

The artwork under study was created in watercolour and India ink on Fabriano paper and belongs to the Hugo Díaz Jiménez's Caricature Collection, stored at the University of Costa Rica's Museum. The bibliography produced in Costa Rica about national cartoon has focused on its semiotic aspect and its ideological background. Thus, the analysis of the caricature's relationship to its socio-political context has been prioritized, while the technical and artistic aspects of the artwork have been neglected. Our research team has been dedicated to the study and preservation of different pictorial works, including oils and watercolours^{11–13}. In the specific case of the latter, we have analyzed five paintings by the Costa Rican artist Fausto Pacheco. For their analysis, multispectral imaging and spectroscopic techniques (X-ray fluorescence, micro-Raman spectroscopy, and scanning electron microscopy-energy dispersive X-ray spectroscopy) were employed to identify the pigments used by the artist. Also, in the case of microbial analysis, non-invasive techniques like swabbing the regions of interest were performed¹⁴. Therefore, examining the composition and conservation state of the support, the colour palettes, pigments and possible biodeterioration of *Cocorí*'s illustration will help define conservation strategies for the future. The conservation of Hugo Díaz's work will allow students, researchers and teachers to continue analyzing the relationship between art, literature, history, communication and social criticism. This is why preserving his work ensures that his critical vision, his artistic quality and his contribution to Costa Rican cultural identity continue to be known and valued, promoting reflection on history, democracy, freedom of expression and the role of art as a tool for social transformation.

2 Results and Discussion

2.1 Visual Description and Multispectral Analysis

The conservation assessment of Hugo Díaz's cover illustration for *Cocorí* was conducted using non-invasive technical photography across the visible (VIS), infrared (IR), ultraviolet (UV), and infrared false-color (IRFC) spectral regions to document its structural condition and creative process.

Visible (VIS) and Infrared (IR) Imaging: High-resolution VIS photography (Figure 2a) mapped surface-level features, highlighting the bright, uniform application of liquid watercolours (Talens Ecoline®) paired with continuous ink lines. In contrast, IR imaging (Figure 2b) successfully penetrated the upper pigment layers to reveal underlying graphite preparatory sketches. In the *Cocorí* cover, the IR reflectography brought to light sub-surface modifications and uninked compositional guidelines in the surrounding flora and character contours, providing direct insight into Díaz's preliminary drafting phase. The infrared (IR) imaging successfully penetrated the upper watercolor layers to reveal Díaz's underlying graphite preparatory sketches and structural modifications. The ability of IR and XRF techniques to expose preliminary drafting phases and obscured compositional changes is highly valuable for understanding the creative workflow of graphic artists, a principle similarly demonstrated in recent synchrotron XRF and IR mappings of obscured paintings and sketches by 19th-century caricaturist Honoré Daumier¹⁵. In the case of the *Cocorí* cover, these uninked guidelines confirm the dual nature of the artwork as both a creative piece and a functional editorial matrix.

Ultraviolet (UV) and False-Color (IRFC) Analysis: UV fluorescence photography (Figure 2c) served to map surface-level interventions and binder interactions; localized areas featuring white corrective media ("patches") reacted by darkening sharply under UV exposure, pinpointing touch-ups made to the original surface. Finally, the IRFC composite (Figure 2d)—generated by remapping the RGB channels between the VIS and IR images (green to blue, red to green, and IR-red to VIS-red)—facilitated a qualitative mapping of pigment distribution, clearly delineating variations in colour density across the cover illustration.

2.2 Priority Attention Zones (PAZ)

The Priority Attention Zones (PAZ) system was established to provide a standardized, quantitative, and spatial framework for evaluating the condition of conservation of the artwork. This evaluation serves as an essential diagnostic baseline for drafting the artwork's conservation condition report and guiding preventive conservation strategies.

The methodology combines systematic macroscopic mapping with a cumulative severity scoring protocol. Macroscopically visible degradation features were recorded and categorized according to established paper conservation terminology¹⁶:

- **Hygro-chemical alterations:** Oxidation/foxing stains, discoloration, water halos (excess water exposure), and intrinsic watermarks (associated with the Fabriano paper substrate).
- **Physical and mechanical alterations:** Mechanical creases or wrinkles, surface abrasion, and graphite residues.

Each recorded alteration feature was cataloged and mapped onto its corresponding grid quadrant (numbered 1 through 100). To quantify the degree of deterioration, numerical severity values were assigned to each instance of damage. The values within each individual quadrant were subsequently summed to establish a cumulative score. Based on these composite scores, a three-tier chromatic scale was developed to classify each quadrant according to its required level of attention: low priority (green), medium priority (yellow), and high priority (red). This score-based classification was then translated into a graphical map to enable rapid visual interpretation of the artwork's conservation needs (Figure 3).

2.3 Fiber density approximation

Characterizing local variations in paper grammage is essential for evaluating structural alterations, mechanical degradation, and non-invasive diagnostic indicators in artworks on paper. Evaluating these localized changes provides quantitative insight into how different degradation mechanisms—such as mechanical stress, chemical oxidation, or material displacement—affect the density and distribution of the paper matrix. To establish this framework, a characteristic intact region was first analyzed to validate the structural parameter estimation against a reference substrate. All reported uncertainties ($\pm$) in subsequent calculated parameters account for statistic measurement errors, using the standard error of the mean. The representative fiber of the paper was characterized from 12 micrographs taken in quadrant 27, see Figure 3, selected for its chromatic variety and absence of visible damage, as shown in Figure 4c. Fiber length and width were measured using *ImageJ* software¹⁷, yielding a mean length of (0.522 ± 0.165) mm and a mean width of (0.018 ± 0.005) mm. Assuming cylindrical geometry, the characteristic fiber volume is $(1.30 \times 10^{-4} \pm 0.04 \times 10^{-4})$ mm³. From the measured artwork dimensions and mass, the quadrant volume is (358.4 ± 11.95) mm³, giving an estimated fiber count of approximately 2.75×10^6 per quadrant and a characteristic fiber mass of $(9.04 \times 10^{-8} \pm 1.81 \times 10^{-11})$ g. The grammage derived from these values, (207.99 ± 0.097) g/m², is in close agreement with the known value of 210 g/m² for Fabriano 5 Watercolour paper¹⁸, validating the approximation.

The simplified single-image method was then applied to five damage regions, as shown in Figure 4d–e and Table 1. The results deviate substantially from the reference grammage in all cases, exceeding 14% in every instance. Despite this, the values remain within the same order of magnitude, and the sign of each deviation provides physically meaningful information about the structural behavior associated with each damage type.

Table 1. Grammage estimates for damaged regions compared to the reference value of 210 g/m².

Damage region	Grammage (g/m ²)	Difference (%)
Wrinkle, quadrant 54	144.17 ± 43.87	−30.7
Oxidation, quadrant 47	237.80 ± 122.61	+14.3
Oxidation, quadrant 9	303.42 ± 84.23	+45.9
Pigment run, quadrant 74	118.27 ± 51.66	−43.1
Graphite trace, quadrant 55	175.34 ± 43.09	−15.7

Regions exhibiting wrinkles and pigment runs yield grammage values substantially lower than the nominal baseline (mean 131.22 g/m²), consistent with either local mass loss or volumetric expansion of the paper substrate induced by the damage. Oxidation stains, on the other hand, yield values exceeding the nominal baseline (mean 270.61 g/m²), suggesting the paper has absorbed mass during oxidation or undergone local compression. A comparison between the two oxidized quadrants is also informative: the stain in quadrant 47, which retains pigment coverage, exhibits a much milder deviation than the bare paper in quadrant 9, indicating that the pigment layer attenuates oxidation over time. The graphite trace in quadrant 55 yields a negative deviation consistent with a slight fiber displacement or volume increase induced by the graphite.

The magnitude of the deviations indicates that a single micrograph is insufficient for a precise grammage estimate. Increasing the number of micrographs per damage region, following the multi-image approach used for the reference quadrant, is the most direct path to improving accuracy. Nevertheless, the directional trends are physically interpretable and sufficient to characterize the structural response of the paper to each type of deterioration.

2.4 X-Ray Fluorescence Spectroscopy (XRF)

Complementary elemental analysis via X-ray Fluorescence Spectroscopy (XRF) was initially performed on pre-selected regions of interest to explore the artwork's chemical composition^{19,20}. However, the elemental analysis primarily provided contextual

matrix information. High-intensity spectral signals were predominantly associated with Calcium (Ca), intrinsic to the paper substrate fillers, and ambient Argon (Ar). The prominence of these peaks partially masked the low-energy lines characteristic of potential metallic pigments. Consequently, the XRF results alone were deemed inconclusive for definitive pigment identification. Therefore, the primary molecular identification of the palette, as well as the evaluation of collateral chemical degradation induced by microbial metabolic activity, were heavily delegated to the multivariate FORS analysis and Principal Component Analysis (PCA) presented in Figure 5.

2.5 Fiber Optics Reflectance Spectroscopy (FORS)

The molecular characterization of both organic and inorganic colorants in the pictorial palette was deepened using Fiber Optics Reflectance Spectroscopy (FORS), an ideal technique for non-invasively recording electronic transitions and molecular absorption bands across a computational grid of 24 Areas of Interest (AOIs) prioritized by their chromatic variance. The resulting raw experimental spectra, mapped in the reflectance versus wavelength plot in Figure 5b, enabled the discrimination of the molecular signatures of ultramarine blue and earth tones like burnt sienna, directly cross-referencing them with the spectral profiles of the Ecoline series by ROYAL TALENS® and reference databases from CHSOS.

The critical contribution of FORS to the interdisciplinary articulation of Figure 5 is evidenced by directly linking the molecular response of the pigments to biological deterioration factors and multivariate statistical processing. In Figure 5b, it can be observed how the reflectance curves of the affected samples display notable morphological distortions and alterations in their slopes, specifically in the critical range from 400 nm to 500 nm. These spectral anomalies do not constitute instrumental noise; instead, they correlate directly with the zones of evident biological growth observed macroscopically on the artwork (Figure 5a) and with the metabolic activity of the microorganism colonies morphologically identified in the culture plates shown in Figures 5e and 5f.

By subjecting this robust set of spectral matrices to the Principal Component Analysis (PCA) illustrated in Figure 5d, the mathematical variance accumulated by the primary principal components (PC1 and PC2) allows for the precise quantification of the vector shifts or *deltas* of the real samples relative to the pure reference centroids. For instance, the anomalous behavior of Sample 4 as an outlier in the blue-spectral quadrant of the PCA is physically justified by the presence of biological metabolic by-products that drastically modify the absorption-reflectance ratio in the visible spectrum. Likewise, the deviations observed in samples such as M1, M2, and M7 toward the spectral coordinates of sienna mathematically reflect the overlapping signatures caused by the physical pigment-mixing technique applied by the artist. This establishes Figure 5 as the core multi-analytical nucleus—integrating chemical, molecular, biological, and statistical data—to define definitive strategies for heritage conservation.

The molecular characterization of the pictorial palette was conducted through Fiber Optics Reflectance Spectroscopy (FORS), a non-invasive technique essential for identifying the electronic transitions of both organic and inorganic colorants. To provide a rigorous spatial framework for this analysis, a computational segmentation was executed using specialized imaging software, identifying 35 specific Areas of Interest (AOIs) prioritized by their chromatic variance and state of conservation.

As illustrated in Figure 2d, the artwork was organized into a systematic grid of quadrants and sub-quadrants. This coordinate-based approach was critical for the precise localization of the artist's characteristic palette—ranging from deep ultramarine blues to earth-based pigments like raw sienna—while simultaneously mapping localized degradation phenomena. This mapping allowed for the correlation of physical wear, chemical shifts, and colour bleeding with specific regions of the composition, facilitating a deeper understanding of the interaction between the medium and the environment.

To ensure a high degree of confidence in pigment identification, the experimental spectra were cross-referenced with a multi-layered reference framework. This included documented data from previous technical studies regarding the artist's historical palette and material preferences, as well as the spectral profiles of the Ecoline series by ROYAL TALENS®. Furthermore, primary reference spectra for pigments such as *Raw Sienna*, *Naples Yellow*, *Ultramarine Blue*, and *Green Gold* were retrieved from the Cultural Heritage Science Open Source (CHSOS) database²¹. This comparative analysis was fundamental in distinguishing between original pigments, the artist's specific mixtures, and the spectral signatures resulting from microbiological or chemical.

2.6 Statistical Analysis: Principal Component Analysis (PCA)

To further investigate the spectral variance and clustering of the identified pigments, a Principal Component Analysis (PCA) was performed on the FORS dataset. This statistical method reduced the dimensionality of the reflectance spectra, allowing for the identification of patterns not immediately apparent in the raw wavelength plots.

As illustrated in Figure 5, the distribution of samples across the PC1 and PC2 axes reveals a coherent sequence following the visible electromagnetic spectrum. The "deltas" (displacements) observed between the experimental samples and the reference centroids (CHSOS and Ecoline) provide critical information regarding the purity and state of the materials, as summarized in Table 2:

Table 2. Multi-analytical correlation of identified pigments, elemental composition (XRF), spectral features (FORS), and statistical status (PCA) for the cover painting of *Cocorí*.

Sample (Area)	XRF Markers	FORS Spectral Features	Suggested Pigment / Mixture	Conservation State & PCA Interpretation
M1, M2 (Brown)	Fe, Mn, Pb (traces)	High absorption in UV-VIS range; spectral curve shifting towards sienna coordinates.	Burnt Sienna / Umber	<i>Stable / Intentional mix:</i> Vector shift away from pure Naples Yellow due to physical palette mixing.
M3 (Blue)	Fe (traces)	Standard characteristic inflection curve of artificial ultramarine.	Ultramarine Blue	<i>Stable / Baseline:</i> Aligns perfectly with the reference standard; baseline cluster in the blue quadrant.
M4 (Blue - Affected)	Fe	Severe morphological distortions and slope flattening in the 400–500 nm range.	Ultramarine Blue (Altered)	<i>Biodegraded state:</i> Positions as a significant statistical outlier due to microbial metabolic by-products.
M7 (Yellow / Cream)		Hybrid inflection with a distinct vector displacement away from the pure reference.	Naples Yellow / Raw Sienna hybrid	<i>Stable / Intentional mix:</i> Reflects complex pictorial technique and tonal saturation variations by Díaz.

- **Spectral Clustering:** Samples 3 and 4 cluster within the blue-spectral quadrant. However, a significant delta is observed for Sample 4, which is positioned as an outlier relative to the Ultramarine Blue reference. This shift is mathematically consistent with the presence of biological by-products, which alter the absorption-reflectance ratio in the 400–500 nm range.
- **Pigment Mixing Deltas:** Samples 1, 2, and 7 exhibit a vector shift away from the pure *Naples Yellow* reference, moving toward the coordinates of *Raw Sienna*. These deltas suggest a complex pictorial technique where Díaz likely mixed pigments to achieve specific tonal saturations, resulting in a hybrid spectral signature.
- **Variance Significance:** The primary principal components (PC1 and PC2) account for the majority of the total spectral variance, confirming that the apparent colour variations are the main drivers of the statistical differentiation, rather than instrumental noise.

2.7 Microbial isolation and characterization

Four out of the twenty sampling sites were colonized with microorganisms. All isolates were retrieved from areas with brown spots or stains. This is in accordance with our previous experience working with microbial colonization of watercolour paintings²². *Staphylococcus epidermidis* was isolated from sampling sites 17 and 18 (quadrants 77 and 80, respectively in Figure 3). This bacterium is a Gram-positive cocci-shaped bacterium that forms part of the normal microbiota of the skin²³; it colonizes high-contact zones of artworks and forms adherent biofilms²⁴. This finding may be relevant as bacteria belonging to the genera: *Microbacterium*, *Staphylococcus*, *Sporosarcina*, *Bacillus* and *Acinetobacter* have been shown to produce organic acids and enzymes with can change the chemical composition of the pigments²⁵.

On the other hand, from sampling sites 7 and 15 (quadrants 26 and 76, respectively) the fungus *Rigidoporus microporus* was retrieved. *R. microporus* is responsible for causing white root rotinfection in plants²⁶ and wood decaying abilities²⁷. This fungus excretes a wide variety of hydrolytic and oxidative enzymes, like cellobiohydrolases and endoglucanases²⁷. It is worth noting that the genera *Rigidoporus* is among the most commonly found fungi in the decaying trees of the Parque Nacional Corcovado, Puntarenas, Costa Rica²⁸. This suggests that this type of fungus may cause deterioration from the cellulose in the drawings.

3 Conclusions

Finally, the main aim of our research was to assess the *Cocorí* cover, a small-scale artwork created for reproduction, from non-destructive techniques which are normally employed in oil or watercolour paintings in order to establish its conservation state over time and ensure it, this caricature is appreciated by future generations. Taken together all the experimental evidence from our study, the results suggest that, firstly, the Priority Attention Zones system focuses our attention on specific regions

of the caricature that can provide evidence regarding the microorganisms, deteriorated region or pigments present. Secondly, non-destructive observation and assessment of the paper grammage can yield reliable information as well as identify areas of significant deterioration. Thirdly, using the baseline information obtained with X-ray and visible-light spectroscopic techniques, we therefore performed multi-analytical correlation which allow us to suggest a pictorial palette comprising the main following pigments: Burnt Sienna or Umber, Ultramarine Blue, Naples Yellow or Raw Sienna hybrid. And fourthly, we successfully isolated two microorganisms belonging to the species *S. epidermidis* (bacterium) and *R. microporus* (fungus). In said matter, a fungi associated with paper degradation was isolated. Its spores, if given the appropriate conditions can germinate and excrete diverse enzymes like manganese peroxidases, laccases and glycoside monooxygenases which will deteriorate the paper. The bacterial isolate is related to manipulation of the artwork, without gloves. This is logically assumed, since its creation, the painting has been handled during the different printing process and was not conceived for decoration or long-term preservation. However, it became an important piece of Costa Rican history and identity, which should be preserved for future generations. Therefore, adequate storage conditions of humidity and temperature will ensure the preservation of this historical treasure.

4 Materials and Methods

4.1 Multispectral Imaging

Photographs were obtained across several regions of the electromagnetic spectrum: visible (VIS), ultraviolet (UV), infrared (IR), and infrared false colour (IRFC), the latter generated computationally from VIS and IR data. The VIS image highlights potential surface deterioration and provides insight into the colours originally applied by the artist, which may have altered over time due to chemical properties or restoration processes²⁹. Infrared imaging uncovers subsurface details of the painting, enabling the visualization of preparatory drawings or modifications introduced by the artist^{30,31}. Ultraviolet photography reveals materials that fluoresce under UV radiation, including certain pigments, binding media, and adhesives^{31,32}.

Multispectral photography was employed, since it is a low-cost technique and the equipment was provided by the UCR Museum. Inspection with UV radiation and VIS spectroscopy was carried out to determine the conservation state of each piece. Technical photographs in the visible and infrared regions, as well as IRFC, allowed a qualitative analysis of the pigments. Finally, pigments were identified using VIS spectroscopy and fluorescence induced by UV radiation.

The required equipment consisted of a professional photographic camera with a modified or full-spectrum sensor. Specifically, a CANON EOS 70D camera with an EF-S 18–55 mm f/3.5–5.6 IS kit STM lens was used. Lighting control was also implemented: for VIS and IR, a halogen lamp of approximately 150 W luminous intensity was utilized, ensuring the same conditions for each photograph. For UV region photographs, a UV radiation source (black light lamps) was employed. The photographs were taken at the university archive (AUROL), since it provided the necessary conditions for proper lighting control. To take the photographs, the piece was placed on an easel at 90°, held at the corners with cured fabric to avoid damaging the integrity of the artwork. The camera was tilted at the same angle as the easel, so that the lens was parallel to the plane of the artwork. In addition, it was positioned at a distance of 11 cm, which is the closest distance at which the camera can focus. The closest possible framing was used to obtain better resolution.

The field of view captured by the camera lens was limited to achieve higher resolution photographs. For this, multiple individual photos were acquired, which were later joined to form a single panoramic image. Each of these photographs had an overlap of 25%–30%, so that when joined, Lightroom® software could merge them seamlessly. ISO values, aperture, and exposure time were optimized experimentally. ISO was kept at 200 to avoid noise in the photograph; aperture was kept as constant as possible, though it varied between spectra; finally, exposure time was adjusted across a wide range of parameters. The second stage corresponded to obtaining the individual images. To avoid vibrations when pressing the capture button, the capture timer was set to 10 s. Additionally, to ensure stability, a tripod was used to place the camera parallel to the plane of the pictorial work. Finally, once the photographs were acquired, digital processing was carried out. Lens corrections, chromatic aberration adjustments, and grayscale conversions were applied to the photos that required them. For this procedure, the following programs were used: Adobe Photoshop® and Adobe Photoshop Lightroom®. Finally, with Adobe Photoshop Lightroom®, panoramas were created by merging all the individual photographs.

4.2 Priority Attention Zones (PAZ)

The spatial distribution of the Priority Attention Zones (PAZ), illustrated in Figure 3, highlights critical correlations between physical deterioration, human exposure, and biological risk across the surface of the artwork.

The analysis revealed that the quadrants requiring the highest level of priority attention (highlighted in red/magenta) are predominantly localized along the outer edges and perimeter of the artwork. In paper conservation, peripheral zones are characteristically the most susceptible to mechanical stress, localized environmental fluctuations, and direct human contact during handling¹⁶. Direct human handling frequently deposits organic contaminants—such as epidermal lipids, proteins, and salts—onto the paper surface, significantly increasing the substrate's bio-receptivity by providing essential nutrients for microfungus growth³³.

Furthermore, these high-risk peripheral zones demonstrated a clear spatial overlap between oxidation/foxing stains, water residue, and microbiological risk. Fungal colonization on cellulosic paper matrices induces enzymatic hydrolysis and oxidative cleavage of cellulose polymer chains, leading to structural embrittlement, local yellowing/browning, and characteristic foxing lesions³³. The co-occurrence of moisture history (water stains) and surface organic contamination along the edges created optimal microenvironmental conditions for active biodeterioration. Consequently, the PAZ map serves not only as a diagnostic summary of physical damage, but also as a predictive risk model for targeted preventive conservation^{16,33}.

4.3 Recognition and characterization of areas of deterioration on the cover of *Cocorí* by Hugo Díaz Jiménez using optical microscopy

To analyze the cover Figure 4a, optical microscopy was employed, as this technique allows observation and image acquisition at magnifications far beyond the capabilities of the human eye. Since the microscope uses only visible light provided by LEDs, it does not cause extensive damage to the artwork, whose pigments are photosensitive and susceptible to degradation under solar and halogen light exposure³⁴.

Total magnifications of 10× and 100× were primarily used while obtaining the images³⁵. The 10× magnification was applied to construct the microscopic panorama Figure 4b, providing a global view of the artwork composed of overlapping microscopic images. This magnification allows for clear identification of deterioration while minimizing the number of micrographs required. The 100× magnification was used to examine specific deteriorated regions in greater detail and to approximate the fiber density of the paper through a series of images taken from a representative area Figure 4c-d. This approach aimed to quantify the extent of deterioration by comparing damaged regions with non-damaged ones Figure 4e.

For the 10× magnification, a ZEISS Stemi 508 stereomicroscope was used, while for the 100× magnification, a Delphi X Observer polarized light microscope for materials science (DX.2058-PLMi) manufactured by Euromex was employed. Both instruments were provided by the Research Center for Microscopic Structures (CIEMic) at the University of Costa Rica, requiring the artwork to be transported from the UCR Museum to the laboratory for image acquisition.

To operate the stereomicroscope, the artwork was placed on the flat stage, the desired magnification was selected, and the focus was adjusted until a clear image was obtained through the software interface. Uniform illumination was achieved using the full LED ring light, and imaging parameters were adjusted, particularly exposure, to ensure consistent brightness across all images. Maintaining a constant exposure through the images, was essential to avoid artifacts when assembling the panorama shown in Figure 4b.

Once all parameters were defined, images were captured and exported, then stored both locally and in the project's cloud repository. Since panoramic reconstruction requires multiple images, adjacent micrographs were overlapped by at least 20% to avoid stitching errors, as illustrated in Figure 4b. Image processing was carried out using Adobe Photoshop 2025, employing automatic alignment and blending functions with appropriate panorama settings³⁶.

For image acquisition with the Delphi X Observer microscope, the slide holders were removed to allow the artwork to rest directly on the microscope stage while remaining as flat as possible. Additional support was used to prevent bending at the edges. After proper placement, magnifications were selected to best visualize deterioration, fibers, or other relevant features Figure 4c-d.

Once the region of interest was properly focused, camera parameters such as white balance, contrast, and gamma were adjusted using the microscope software to achieve the highest possible image quality. The images were then captured, annotated with relevant measurements, and stored in the personal cloud.

Once the images have been obtained, the corresponding procedure is carried out, beginning with a description of the damage to the paint layer. Micrographs of damage were analyzed to identify their microscopic characteristics. Potential causes of deterioration include physical impacts, natural degradation of photosensitive pigments, exposure to environmental agents such as humidity or air, contamination from public handling, or the presence of microorganisms.

Next, an approximation of fiber density was obtained from a set of images taken within a single quadrant, and the result was extrapolated to the entire artwork Figure 4c. This analysis was conducted using ImageJ software¹⁷. The image scale was defined, and fibers located within the same focal plane were identified and marked. Fiber length and width were measured, with the width calculated as the average of three measurements along each fiber. These values were averaged to define a representative fiber, assumed to have a cylindrical geometry¹⁸.

Once the dimensions of the representative fiber were determined, the mass, length, width, and thickness of the artwork were measured, allowing the corresponding values for the selected quadrant to be calculated. From these data, the number of fibers and the volumetric fiber density were estimated. This value was used to calculate the paper grammage, which was compared with the known theoretical value for the specific paper type to validate the approximation¹⁸.

Finally, a simplified method was applied to estimate fiber density from a single image of a damaged area Figure 4d. Fiber dimensions were measured to define a representative damaged fiber, and the mass was estimated based on the general representative fiber. The number of fibers within the image was approximated, allowing calculation of fiber density in the

damaged region. This value was compared with the general fiber density to determine whether deterioration is associated with changes in density Figure 4e.

4.4 FORS and XRF Setup

To identify and select areas with deterioration, a preliminary image processing step is required. Adobe Photoshop 2022 was employed, applying the “auto-align” and “auto-blend” functions with parameters adjusted for panoramic correction. Once these adjustments were completed, it was possible to sample regions potentially affected by oxidation, folds, or other types of damage.

The FORS system, employed for non-invasive sample analysis, consists of a light source, optical fibers, a sample holder, and a spectrometer. The light source, typically a xenon or tungsten-halogen lamp, illuminates the sample across a broad wavelength range, usually from UV to NIR. Optical fibers transmit the incident light to the sample and collect the reflected radiation, which is then directed to the spectrometer for analysis. The spectrometer records the intensity of reflected light at different wavelengths, producing a reflectance spectrum for the sample. By analyzing this spectrum, it is possible to identify features corresponding to specific pigments or materials. The reflectance data are then compared with spectral databases of known pigments, enabling the identification of materials present in the artwork without the need for invasive sampling. In this study, fiber optics reflectance spectroscopy was performed using the high-resolution FieldSpec® 4 Hi-Res spectroradiometer (ASD brand). The results were stored in the RS3 3D Finite Element Analysis software, from which the data could be exported to ViewSpecTMPPro for further processing.

Data processing and graphical representation were executed through custom scripts developed in MATLAB. This computational approach allowed for the precise plotting of reflectance curves and the statistical treatment of the spectral data. To ensure a reliable identification of the materials, the experimental results were compared against the Cultural Heritage Science Open Source (CHSOS) reference database. This comparative analysis facilitated the correlation between the observed spectral features and the known signatures of historical pigments and substrates.

The experimental technique applied is X-ray fluorescence spectroscopy (XRF), which directly refers to the observation and study of the spectrum based on the interaction between radiant energy and matter³⁷. This method enables both qualitative and quantitative analysis of materials and their composition.

Specifically, the technique operates through the emission of high-energy photons from an X-ray tube. These primary X-rays irradiate the sample, exciting the electrons present in the artwork. As a result, the electrons emit secondary X-rays at discrete energy levels, known as X-ray fluorescence.

The generation of the spectrum arises from the emission of these secondary X-rays, which appear as peaks in the spectrum corresponding to discrete energy values. By measuring the energy, it is possible to identify colours associated with specific chemical elements. Likewise, by measuring the intensity of the emitted energy, the quantity of each element can be determined, represented in the spectrum as peak intensity¹⁹.

The direct application of this technique to the object of study allows the identification and quantification of constituents in diverse samples, in this case pigments in artworks. In this research, X-ray spectroscopy was specifically employed, operating in the region to the left of the visible spectrum.

Another fundamental aspect for understanding XRF spectroscopy is that many substances exhibit fluorescence. This means that once irradiated, the substance has the ability to emit light. As noted by Heredia³⁸, fluorescence occurs when a substance emits visible light after absorbing radiation of a shorter wavelength. It is important to highlight that fluorescence differs from phosphorescence in terms of process speed, with fluorescence being faster.

In addition, computational analysis techniques provide preliminary insights into spectral results. Once the regions of interest in each artwork are selected, two computational programs are used to obtain intensity values and classify pigment probabilities.

The first program, developed in Python, implements the *Intensity Colors* code, which relates colour intensity in specific or selected regions that may be relevant for study. The second program, *Golden Artist Colors*, is freely accessible online. Once the image is uploaded, the system allows the selection of regions or points of interest, automatically generating several possible pigment identifications for the chosen area. This tool is essential for providing preliminary results that can later be confirmed or refuted through spectral analysis. Figure 3 shows a descriptive image of the Golden Artist Colors interface.

Based on the description and development of these techniques, the main results are presented below, further detailing the application and methodological framework of each technique.

4.5 Microbial isolation and characterization

Twenty samples were collected using dry sterile swabs that were rubbed over the sections of interest. After the collection, the swabs were immersed in 5 mL of 0.1% Sterile Peptone Water and then transported at 4 °C to the laboratory for further analysis. The swabs were used to inoculate Blood Agar (BA) plates for bacterial isolation, and Sabouraud glucose agar (SGA) for fungal growth. The BA plates were incubated for 48 hours at (35 ± 2) °C. After incubation, bacterial colonies were microscopically analyzed with Gram staining and identified using the VITEK® 2 System (bioMérieux, Marcy-l'Étoile, France). The SGA

plates were incubated at $(25 \pm 2) ^\circ\text{C}$ for three weeks. Subcultures of the different colonial morphotypes were performed on Potato Dextrose Agar. The purity and preliminary identification of the isolates were determined using cotton blue lactophenol dye. Fungal identification was performed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using a MALDI Biotyper system (Bruker Daltonics, Billerica, MA, USA). The spectra were analyzed using the Bruker Library and the MSI (Mass Spectra Identification) platform³⁹.

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Author contributions

J.G.B.C., R.M.D.I.P., and D.J.V. designed the methodology, accomplished the majority of the experiments, and conducted the acquisition and analysis of the main results. The complete microbiological study was outlined and fulfilled by D.J.V., A.G.A., S.L.A., and M.R.S. J.G.B.C. carried out all the characterization of the deterioration of the studied pictorial artworks. L.R.C. enhanced the information regarding the conservation aspects and the historical context of the artist and his artwork.

524 A.M.R.G., M.C.C., and M.R.S. contributed to data analysis and experimental validation. J.G.B.C. prepared the final version
525 of all figures. The manuscript was drafted and edited by all the co-authors. O.A.H.S. formulated and guided the whole
526 project. O.A.H.S. was involved in experimental design, data analysis along with interpretation, and conclusions. All co-authors
527 discussed and commented on the main results presented in the manuscript. All authors have given approval to the final version
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531 **Competing interests**

532 The authors declare no competing interests. O.A.H.S. declares that he is a Editorial Board Member of Nature Portfolio journals;
533 furthermore that the content of this manuscript is the original work of our research group, it has not been published previously
534 in any journal, and it is not being considered or informed by any editorial office.



Figure 1. This image is a reinterpretation of the illustration created by Hugo Díaz for the book *Cocorí*. It illustrates the scientific study process explained in this article while simultaneously paying homage to Díaz's work. The proposal takes elements from the original illustration and transforms its characters into the protagonists and leaders of the scientific study conducted on the artwork. Thus, the character of *Cocorí* becomes a scientist exploring himself through X-rays. This reinterpretation seeks to preserve the spark, imagination, and playful dimension of the original piece. Figure elaborated by Guarida Studio (Paula Méndez & Jeff Badilla).



Figure 2. Multispectral imaging of the *Cocorí* cover. (a) Visible light. (b) UV fluorescence (microbiological zones). (c) IR imaging (XRF zones). (d) IRFC (FORS zones).

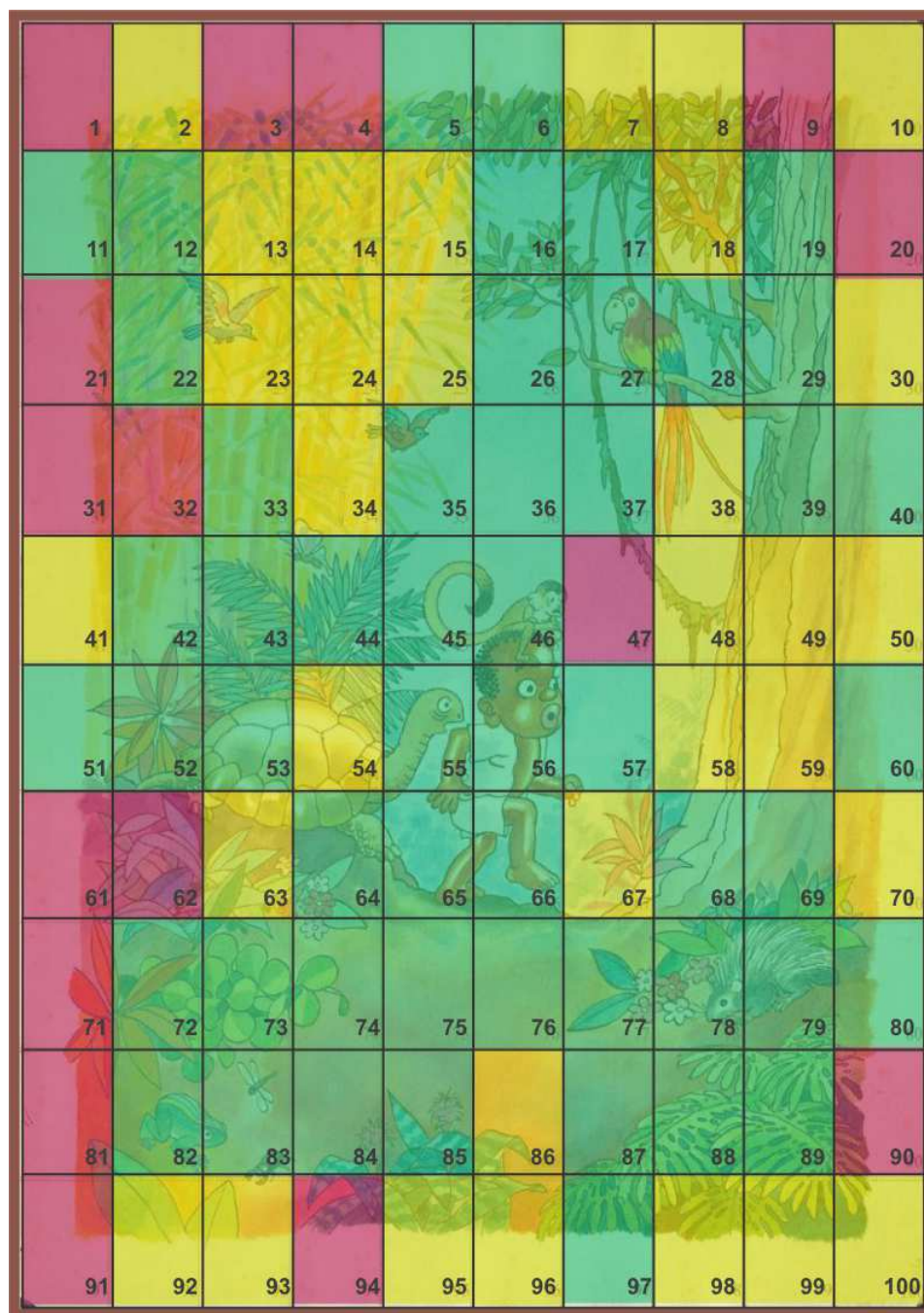


Figure 3. Priority Attention Zones. This figure evinces the priority level of attention according to the physical deterioration present in each quadrant of the artwork. The priority, in ascending order, is represented by the colours: green for low, yellow for medium, and red for high.

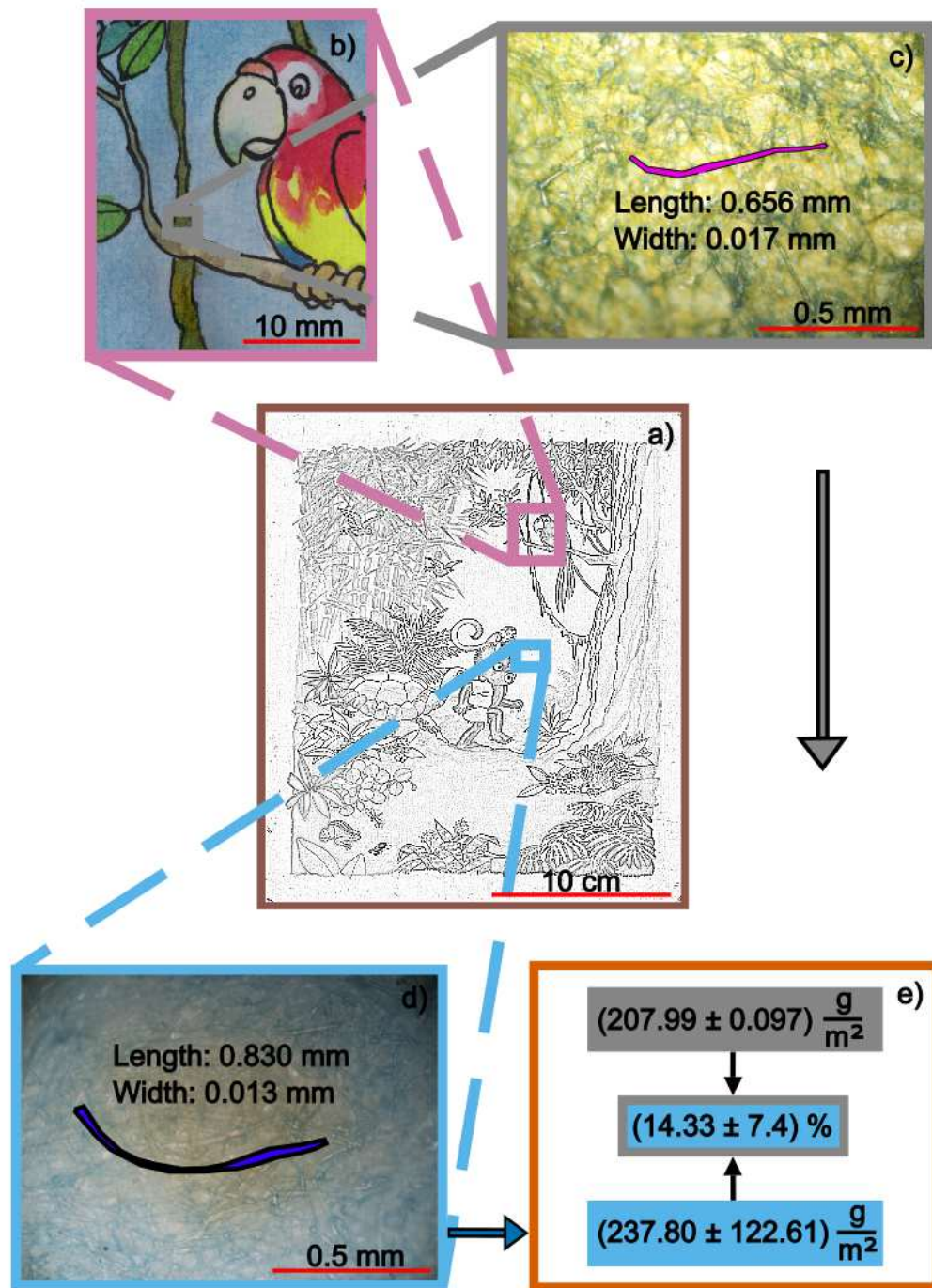


Figure 4. Recognition and characterization of areas of deterioration on the cover of *Cocorí* by Hugo Díaz Jiménez using optical microscopy: (a) Original cover. (b) Create a partial panorama of the cover using at least 15 microphotographs overlapped by 20%, and identify the zones of interest. (c) Calculate the average dimensions of the healthy fibers based on measurements from 8–13 fibers obtained from 12 different microphotographs. Then calculate the healthy fiber density using the previous data. (d) Calculate the average dimensions of damaged fibers using 10–13 measurements from one microphotograph per damage type. Then determine the damaged fiber density and compare it with the healthy fiber density. (e) Compare the results from the damaged fibers with the healthy ones expressing it as a percentage of difference.

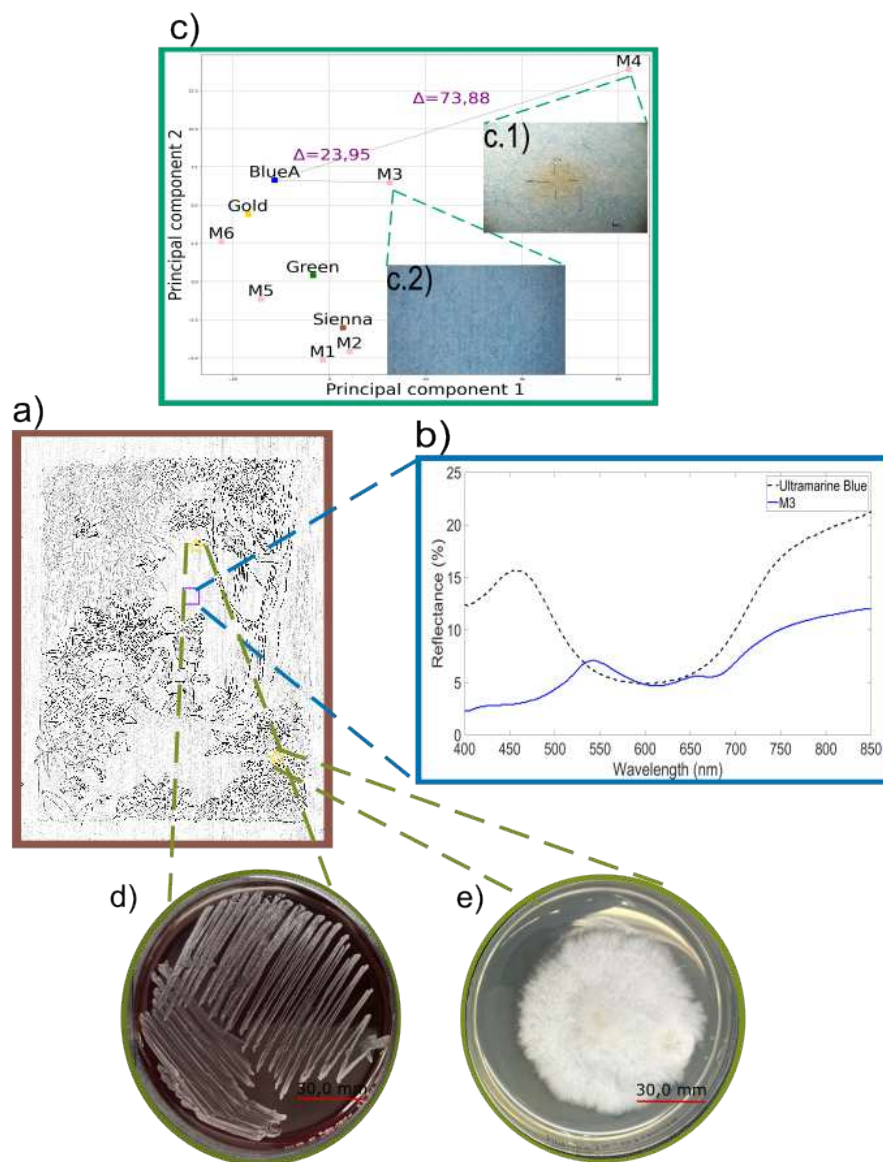


Figure 5. Comprehensive spectral, statistical, and microbiological characterization of artwork samples. (a) Representative surface/microscopic mapping grid indicating feature distribution across analyzed coordinates. (b) Fiber and pigment FORS reflectance spectra comparing reference Ultramarine Blue against sample M3 across the 400–850 nm wavelength range. (c) Principal Component Analysis (PCA) score plot illustrating spatial clustering for pigment and damage samples M1–M6 (grouping Sienna, Green, and Gold regions), with embedded high-magnification optical micrographs showing representative damage zones (**c.1**: localized degradation spot with measured area parameter $\Delta = 73.88$; **c.2**: uniform pigment matrix area with $\Delta = 23.95$). (d–e) Isolated microbial morphotypes cultured from high-priority deterioration zones, shown with 30.0 mm scale reference bars.