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An analytical study of the Huexotzinco Codex using X-ray fluorescence, fiber optic reflectance spectroscopy, and portable Fourier-transform infrared spectroscopy

Tana Elizabeth Villafana[1*], Mary Elizabeth Haude[2] and Amanda Satorius[1]

Abstract

The Huexotzinco Codex is one of the earliest surviving manuscripts from the early colonial period of Mexico. The codex pertains to the legal case of conquistador Hernán Cortés and contains paintings and documents detailing the seizure of Cortés' personal properties and over-taxation of his Huexotzincan allies by the colonial government in New Spain (present-day Mexico). Eight paintings within the manuscript were subjected to non-invasive analytical techniques revealing the pigment palette and production methodology. The findings of this study show a mixture of pre- and colonial codex production practices as well a unique Maya blue formulation and the identification of a silicate material used as a matrix for an organic yellow dye.

Keywords: Mesoamerican codices, Huexotzinco Codex, Fiber optic reflectance spectroscopy, X-ray fluorescence spectroscopy, Portable infrared spectroscopy, Non-destructive analysis, Pigment identification

Introduction

Mesoamerican codices are the best primary sources for understanding the historic and cultural legacy of the ancient Nahua (which includes the Mexica, or Aztecs) and Mayans. There has been on-going research for the past decade or more devoted to the non-invasive analysis of pre- and colonial codices in an attempt to better understand not just the materiality of these works but also technological similarities between different Mesoamerican cultural traditions, as well as changes introduced by the Spanish colonizers. Studies of codex materials [1–5] thus far have uncovered region-specific pigment and papermaking technologies and experimentation, revealed important iconography, and provided historic details about life and culture before and after the Spanish invasion. Changes in materials and production of the codices also show the growing influence of European painting techniques and pigments.

The Huexotzinco Codex is significant as it is one of the earliest surviving manuscripts from the early colonial period of Mexico. Produced in 1531 in the town of Huexotzinco, Puebla, the codex consists of eight pictographic paintings and 79 documents written in Spanish concerning the legal case of conquistador Hernán Cortés against three members of the First Audiencia, the colonial government in New Spain. The eight paintings of the codex were created by three Indigenous scribes, or *tlacuiloque* in the Nahuatl language, and represent pictorial testimony about the seizure of Cortés' personal property [6, 7]. The paintings reflect a predominantly Indigenous tradition with regard to their convention of communicating information through pictographs and their use of Indigenous papers and colorants.

Despite the historic importance of this manuscript, the Huexotzinco Codex has not been the subject of an

*Correspondence: tvil@loc.gov
[1] Preservation Research and Testing Division, Library of Congress, 101 Independence Ave. SE, Washington, DC 20540, USA
Full list of author information is available at the end of the article

extensive analytical study. The provenance of the codex is fairly well-documented [7]; United States philanthropist Edward Stephen Harkness acquired the codex from the US book dealer A.S.W. Rosenbach who had previously purchased it from a descendant of Cortés. Until 1925, the codex was part of the private archive of the Italian dukes of Monteleone, descendants of Cortés, and housed in the archives of the Hospital de Jesús de Nazareno in Mexico City. Harkness donated the Huexotzinco Codex to the Library of Congress (LC) in 1928, where it currently resides in the Manuscript Division's Harkness Collection. The fragile condition of the manuscript prevented it from being studied by scholars until Sylvia Albro began conservation treatment in 1986 (detailed in [8]). During the conservation treatment, fiber and pigment samples were taken from the eight codex paintings to perform limited material analysis using polarized light microscopy (PLM). A significant contribution Albro made to the understanding of the material aspects of the Huexotzinco Codex, and Mesoamerican codices, was her identification of two types of Indigenous paper, maguey and amate [8]. She also identified charcoal as the black pigment used through-out the manuscript and cochineal as the red on several of the paintings. In a later collaboration, Albro and Mary Elizabeth Haude, reviewing the pigment samples using PLM, identified Maya blue and Maya green (personal communication).

The goal of this paper is to expand upon the earlier analysis and to study the materiality of the eight codex paintings in-depth using a variety of non-invasive techniques, including X-ray fluorescence (XRF) spectroscopy, fiber optic reflectance spectroscopy (FORS), portable external reflectance Fourier-transform infrared spectroscopy (ER-FTIR), and microscopy. This research can also be placed into context with other studies performed on codices from similar regions and time periods [9–12], such as the codices Borgia, Cospi, Fejérváry-Mayer, Laud, Vaticanus B, and Mendoza, in order to add further insight into the cultural traditions of codex production and early European influences.

Methods

X-ray fluorescence spectroscopy

X-ray fluorescence spectroscopy (XRF) was performed with a Bruker Tracer III. The portable spectrometer has a Rh tube excitation source, 8 μm Beryllium detector window, and a proprietary silicon drift detector. Each spectrum was collected with the X-ray source operating at 20-μA and 50 kV, in air, with a data integration time of 30 s. Spectra were collected by securing the codices on a backless easel with the Tracer III held roughly 1 cm above the point of interest, with a spot size of 3 mm. The configuration of the codices on the easel ensured the XRF spectra only contained information from the codices. The instrument is controlled, and data analyzed using Bruker software. Spectra were normalized to the Compton peak to allow for qualitative comparison between relative peak intensities between the different codices.

Fiber optic reflectance spectroscopy

Fiber optic reflectance spectroscopy (FORS) was undertaken using an ASD FieldSpec 4 Hi-Res spectroradiometer (Malvern Analytical). The spectral resolution is 3 nm at 700 nm and 8 nm at 1400 and 2100 nm. Light from a halogen bulb (ASD Contact Probe) was used as an illumination source held at a 45° angle and a height of roughly 20 cm from the surface. The detection fiber (separated from the contract probe, because non-contact measurements were preferred) was held perpendicular to the surface at a height of roughly 1 cm, giving a 3 mm sampling size. Ten spectra were averaged for each region of interest with a total acquisition time of < 5 s. Spectra were normalized to a Spectralon white reference standard. The system is controlled via ASD proprietary software, RS^3. Reflectance spectra were viewed and otherwise processed using ViewSpec Pro; first derivatives were calculated using a built-in ViewSpec Pro software function with a derivative gap of nine to highlight the rate of change of the reflectance with respect to the wavelength.

Fourier-transform infrared spectroscopy

A Bruker ALPHA portable FTIR spectrometer with a KBr beamsplitter, a DGTa detector and an external reflectance (ER-FTIR) module with a gold reference standard was used mounted on a tripod with no aperture for complete no-contact analysis. Each spot was analyzed using 64 scans from 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1}. Spectra were collected and viewed in Bruker's proprietary software OPUS Video 7.4.

Brightfield and fluorescence microscopy

Microscopy was performed using an AxioImager.D1m (Carl Zeiss) with white light (halogen lamp, HAL100, Zeiss) and UV (mercury lamp, HBO100, Zeiss) in conjunction with a turret cube for UV eye protection (Chroma). Two objectives were used, LD plan-Neofluar 20×/0.4 KORR with adjustable collar set to zero (no coverslip), and EC Epiplan-Neofluar 50×/0.8 DIC M27. The eye pieces provide an additional 10× magnification. Images were captured using an AxioCam HRc CCD and processed with AxioVision software.

Results

Each painting was examined using XRF, FORS, ER-FTIR, and microscopy. Painting VI (Fig. 1) is colorless, hence, only XRF and FORS were performed on the paper.

Fig. 1 Digital photograph of Painting VI, the only colorless painting in the codex

A brief summary of the material analysis is shown in Table 1, which includes the paper support (as identified by Albro [8]) and identified colorants for each painting.

Paper

The FORS spectra seen in Fig. 2 are typical for cellulose based paper. The spectral features in the range from 1000 to 2500 nm originate from vibrations in carbon, hydrogen, and oxygen bonds absorbing particular wavelengths of light. These vibrational energies have been previously assigned [13], however, Paintings I, IV, and VI (on amate paper) have spectral features in the 1400–1500 nm and 1900–2100 nm range that are not related to cellulose. These peaks, more obvious in the FORS 1st derivative features at 1453, 1955, and 1993 nm, are associated with the presence of gypsum [14, 15]. The XRF spectra of these same codex paintings are the only ones that show high amounts of calcium (Ca) and sulfur (S). Elevated amounts of potassium (K) appear to correlate with higher intensities of Ca and S, however the exact reason for this is unknown. The spectral differences between the amate and maguey paintings points toward the two types of paper having a different fiber preparation technique making it quite possible that K is either a purposeful additive or a mineral impurity occurring during the preparation of amate. The concurrence of several FORS features associated with gypsum with relative elevated intensities of Ca and S in XRF seems to indicate a trace amount of gypsum in the paintings on amate paper. No gypsum related peaks were found on any of the paintings on maguey paper.

Microscopic examination using the fluorescence modality revealed that maguey papers have a much higher intensity of fluorescence (camera exposure time was set roughly 10× longer in the amate fiber image in comparison to the maguey), and although subjective, appears to be a different color. Lignin, unlike cellulose, fluoresces blue when excited with ultraviolet light [16, 17]. López Binnqüist et al. [18] found that traditional amate paper, made from the inner bark of the Ficus tree, from the pre-Hispanic period was naturally lignin free, whereas maguey fibers have a high (though varying) lignin content [19]. Therefore, the natural lignin content is a possible explanation of the fluorescence differences in the two papers.

Table 1 Summary of material analysis

Painting	Paper	Black	Red	Purple	Orange	Blue	Green	Yellow
I	Amate	Carbon	Cochineal	Cochineal + carbon black	Iron-rich Kaolinite			
II	Maguey	Carbon	Cochineal		Iron-rich Kaolinite			
III	Maguey	Carbon	Cochineal			Maya blue	Maya blue + organic yellow	Organic yellow-diatom hybrid
IV	Amate	Carbon	Cochineal					
V	Maguey	Carbon	Cochineal		Iron-rich Kaolinite	Maya blue	Maya blue + organic yellow	Organic yellow-diatom hybrid
VI	Amate	Carbon						
VII	Amate	Carbon	Cochineal		Iron-rich Kaolinite			
VIII	Maguey	Carbon	Cochineal	Cochineal + carbon black				

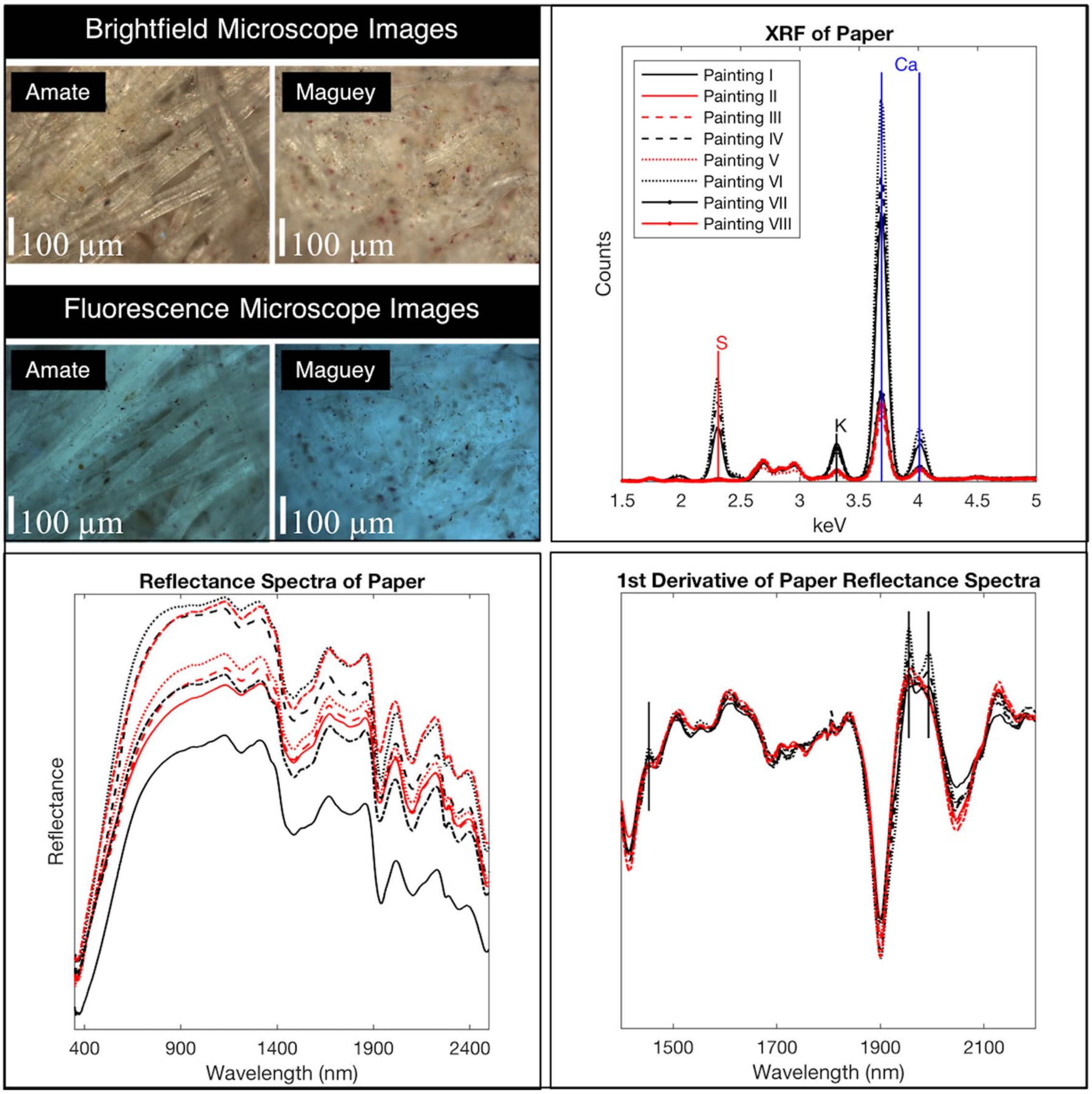

Fig. 2 Microscopic images, XRF, and FORS of amate and maguey papers. Microscope images were taken under a halogen and mercury lamp with a ×20 objective. Gypsum related peaks are marked in the XRF (S, K, and Ca) and FORS 1st derivative spectra (1453, 1955, and 1993 nm)

Pigment identification

FORS of the red colorant across seven of the codex pages show absorption features at 485, 525, and 565 nm with 1st derivative peaks at 495 and 535 nm, spectral features consistent with an insect-sourced anthraquinone dye [20–22], such as kermes, lac, or carmine cochineal. Although these three dyes cannot be distinguished using FORS alone, it is presumed the red colorant is carmine cochineal based on historical sources [23], identification of cochineal on a majority of Central Mexican and Oaxacan manuscripts [9, 11, 24, 25], and the previous study and treatment by Albro [8], during which she compared known cochineal samples to samples from the Huexotzinco Codex using polarized light microscopy.

According to recent research [22], these spectroscopic features indicate that the cochineal on each of the Huexotzinco Codex paintings is likely an alum-based cochineal lake. Further, the XRF baseline spectrum of the paper was compared to the paintings' red painted areas, and these areas were found to have elevated levels of S and K, corroborating the use of an alum mordant. Figure 3 shows a representative reflectance spectrum from one area of red coloration from each of the seven codex paintings, along with their 1st derivative, as well as XRF

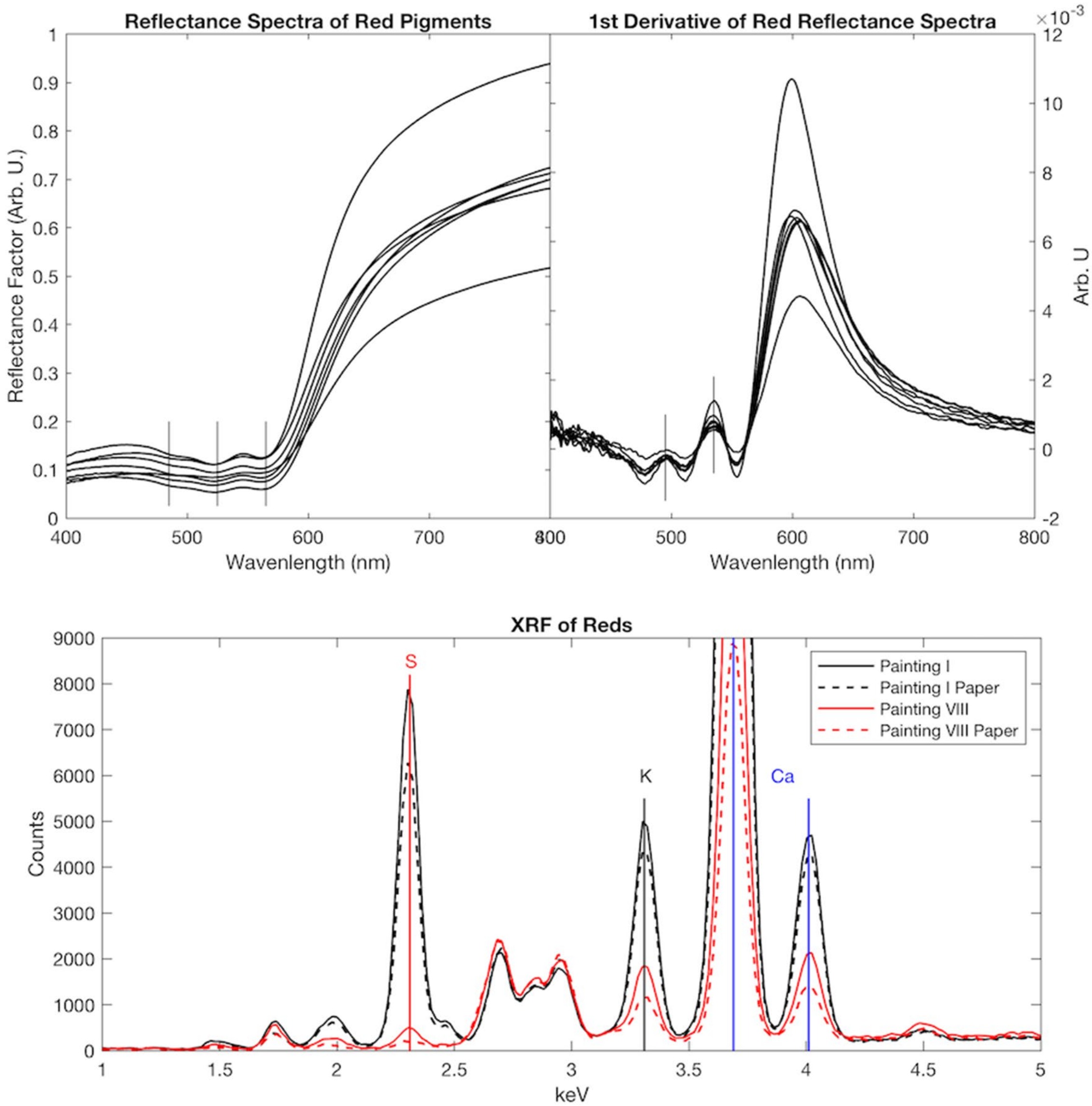

Fig. 3 FORS and XRF confirming the use of an alum-based cochineal lake as the red colorant on the codex paintings. Spectral features relating to the cochineal lakes are marked in the reflectance spectra (485, 525, and 565 nm), the 1st derivative spectra (495 and 535 nm), and the XRF (S, K, Ca)

spectra from Paintings I and VIII to highlight the elevated S and K signal counts of red areas in comparison to amate and maguey paper support.

Historic research states that the town of Huexotzinco (from which the codex originates) was one of the main townships for cochineal cultivation in Central and South America from pre-Hispanic times until the end of the sixteenth century [23, 26, 27]. The spectroscopic similarity between the red colorants used in these seven codex paintings (Fig. 3) indicates that either the production method of cochineal was highly standardized or that the scribes shared paints. Both theories are supported by research; Indigenous scribes are known to have shared pigments [28] and it is well documented that painters could acquire cochineal from markets in the form of dried cakes called *tlaxcalli* in Nahuatl [24]. The selling of *tlaxcalli* in a marketplace indicates a standardized production method and somewhat mass production of cochineal, making it a reasonable assumption, although still conjecture, that the scribes used pigments from the same market.

The spectral and visual similarity of the cochineal lake is even more striking when considering the extreme sensitivity of the dye to the extraction and laking process [20, 29, 30]. Figure 4 shows Paintings IV and VII, for visual examples of the rich, red cochineal used through-out the codex paintings and microscopic examination found uniform particles. The lack of fluorescence under the mercury lamp further points to a unique and well-defined production practice. While certain mordant substrates are known to quench the fluorescence of cochineal, alum is not among them [31]. Red dyes are known to undergo a self-absorption phenomenon [20, 32], however, this is typically characterized by a red-shift in the fluorescence emission wavelength rather than total fluorescence quenching. Further work is required to fully understand the production method of cochineal in ancient Mesoamerica.

Due to the abundance and popularity of cochineal, it was common practice to mix or dilute the rich red pigment to create other colors, such as purple and orange [24]. Microscopic examination revealed that areas appearing visually purple in Paintings I and VIII are a mixture of carbon black and cochineal, as seen in Fig. 5. Although the presence of a black pigment dramatically lowers the reflectance intensity from purple areas, the absorption and 1st derivative features in purple areas (Fig. 5) were identical to the alum-based cochineal lake used in areas of pure red. However, Paintings I, II, V, VII, and VIII have areas painted with a stand-alone orange.

Identification of the orange pigments as an iron-rich kaolinite was confirmed by FORS, ER-FTIR, and XRF (Fig. 6). Kaolinitie is typically associated with the elements silicon (Si), titanium (Ti), iron (Fe), and K, which are present in the XRF spectrum of each orange painted area. Trace amounts of aluminum (Al) were detected, but as a light element, the peaks have typically very low intensity in X-ray spectra. FORS confirmed the Fe(III) ion by absorption features at 650 and 910 nm [14, 21, 33] and kaolinite by absorption features at 1391 and 1414 nm (OH and molecular water vibrations [34]) and 2158 and 2205 nm (Al–OH vibrations [14, 34]). The vibrational energy of silicates and hydroxides associated with kaolinite [12] are also confirmed with ER-FTIR, showing peaks at 1047, 1004, 540, and 468 cm^{-1} (silicates) and 3698 and 3622 cm^{-1} (hydroxides).

Given that the Huexotzinco Codex is one of the earliest colonial manuscripts, the identification of this inorganic

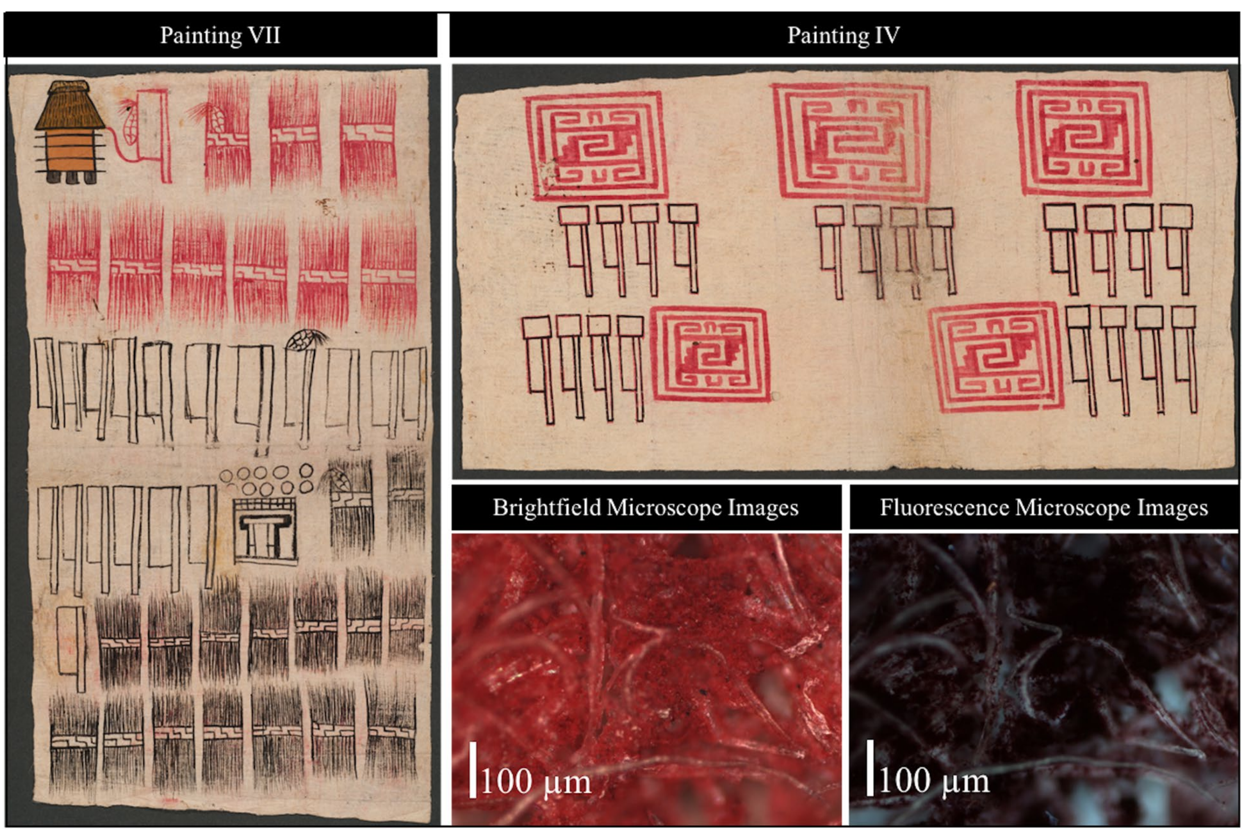

Fig. 4 Digital photograph of Paintings IV and VII show examples of red painted areas. Microscope images were taken from Painting VII using a halogen and mercury lamp with a ×20 objective. The cochineal was found to be non-fluorescent

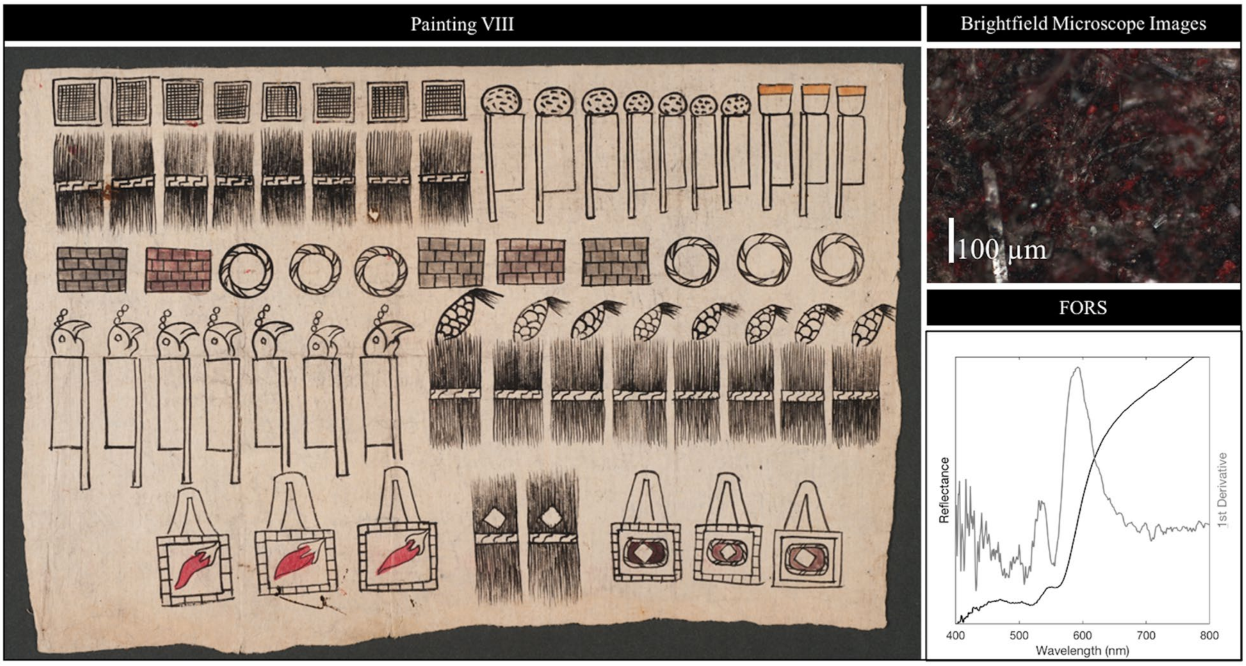

Fig. 5 Digital photograph of Painting VIII, microscope image of purple bricks from Painting VIII and the respective FORS analysis. Absorption features at 485, 525, and 565 nm with 1st derivative peaks at 495 and 535 nm are consistent with an alum-based cochineal

orange pigment is surprising. Recent scholarship suggests the use of mineral pigments was due to European influence [35] as mineral pigments were rarely used on pre-Hispanic Nahua codices. The exception has been the finding of orpiment on the pre-Hispanic Codices Cospi, Fejérváry-Mayer, Laud, and Nutall [9–11, 35]. Otherwise, iron-based earth and clay mineral pigments have only been identified on later sixteenth-century Mexican manuscripts, such as the Codex Mendoza [11, 35], the Florentine Codex [1, 36], and the Relaciones Geografícas maps [37]. Kaolinite is an aluminum silicate clay and is naturally abundant in many areas of Mexico. A large mineral deposit in Puebla, Mexico consists mainly of kaolinite that contains various amounts of silicates, titanium, and iron oxides [34]. Kaolinite was not commonly used in Nahua codex production, and the presence of orange kaolinite, the only inorganic pigment on the Huexotzinco Codex is unclear, as the other pigments are either organic dye-based colorants and organic–inorganic hybrid pigments. Its use could be due to European influence, or it could be the result of the changing society and the changing role of Indigenous books and manuscripts in early colonial Mexico [35, 38]. However, its presence on the codex paintings, which were used as evidence in a court case, fits with what Domenici described as the evolution of material usage and artistic practices by Indigenous artists to communicate with new audiences in the colonial period, such as courts of law [35].

Paintings III and V are the only paintings featuring colorants that are yellow, blue, and green. Results from Painting III are seen in Fig. 7. In blue pigmented areas, FORS shows increased reflectance between 400 and 530 nm with the main absorption features at 648 nm. The ER-FTIR spectrum has features due to OH and Si–O vibrations associated with sepiolite (426, 653, and 3675 cm^{-1}), palygorskite (509, 3550, and 3601 cm^{-1}), and features common or intermediate to both (1029, 978, and 467 cm^{-1}). These spectral features are indicative of Maya blue, an indigo-clay hybrid pigment unique to Mexico and Central America and widely used in ancient Mesoamerica.

Modern studies on Maya blue [39–42] elucidated that Maya blue is a nanostructured, hybrid organic–inorganic material composed of indigo incorporated into a fibrous phyllosilicate clay, palygorskite, upon heating. This indigo-palygorskite hybrid has been accepted as the “proper” form of Maya blue. Recent research on a range of Mesoamerican codices has shown that Maya blue can be further subdivided into different groups. These subclasses (Maya blue created using either low indigo content or very high heating temperatures with palygorskite, a sepiolite-indigo hybrid, or simply as indigo dye supported on clays) can be distinguished using FORS and

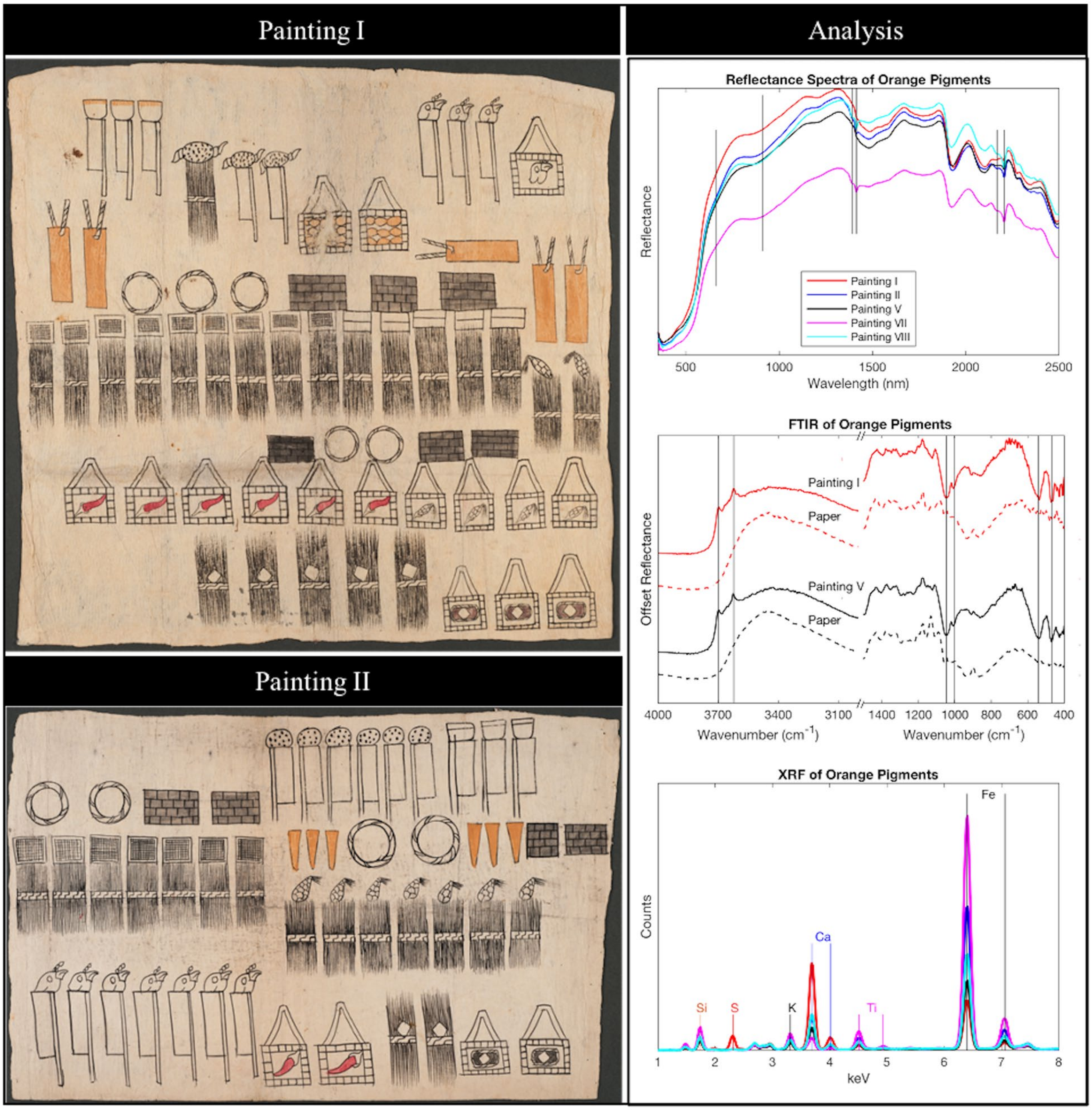

Fig. 6 Digital photographs of Paintings I and II show areas of the orange pigment, along with FORS, ER-FTIR, and XRF analysis. Spectral features related to kaolinite are marked in the FORS (650, 910, 1391, 1414, 2158, and 2205 nm), ER-FTIR (3698, 3622, 1047, 1004, 540, and 468 cm^{-1}), and XRF spectra (Si, S, K, Ca, Ti, Fe). ER-FTIR shows the paper substrate along with a spectrum of the orange area on Painting I (amate) and V (maguey) to represent both paper types

ER-FTIR [12]. Using the spectral features described by Grazia et al., the Maya blue in Painting III (and Painting V, results in Fig. 8) can be characterized as a unique formulation in which an indigo-clay hybrid was created using a mixture of palygorskite and sepiolite; absorptions below 650 nm are indicative of an indigo-sepiolite hybrid while the reflectance features are more in line with a "proper" Maya blue. ER-FTIR confirmed the presence of palygorskite and sepiolite. Of the thirteen codices studied [12] that were painted with Maya blue, only the Codex Borgia [43] was found to have a Maya blue created with a mixture of the two clays palygorskite and sepiolite. Although the exact formulation of the Maya blue in the Huexotzinco Codex and Codex Borgia are not identical,

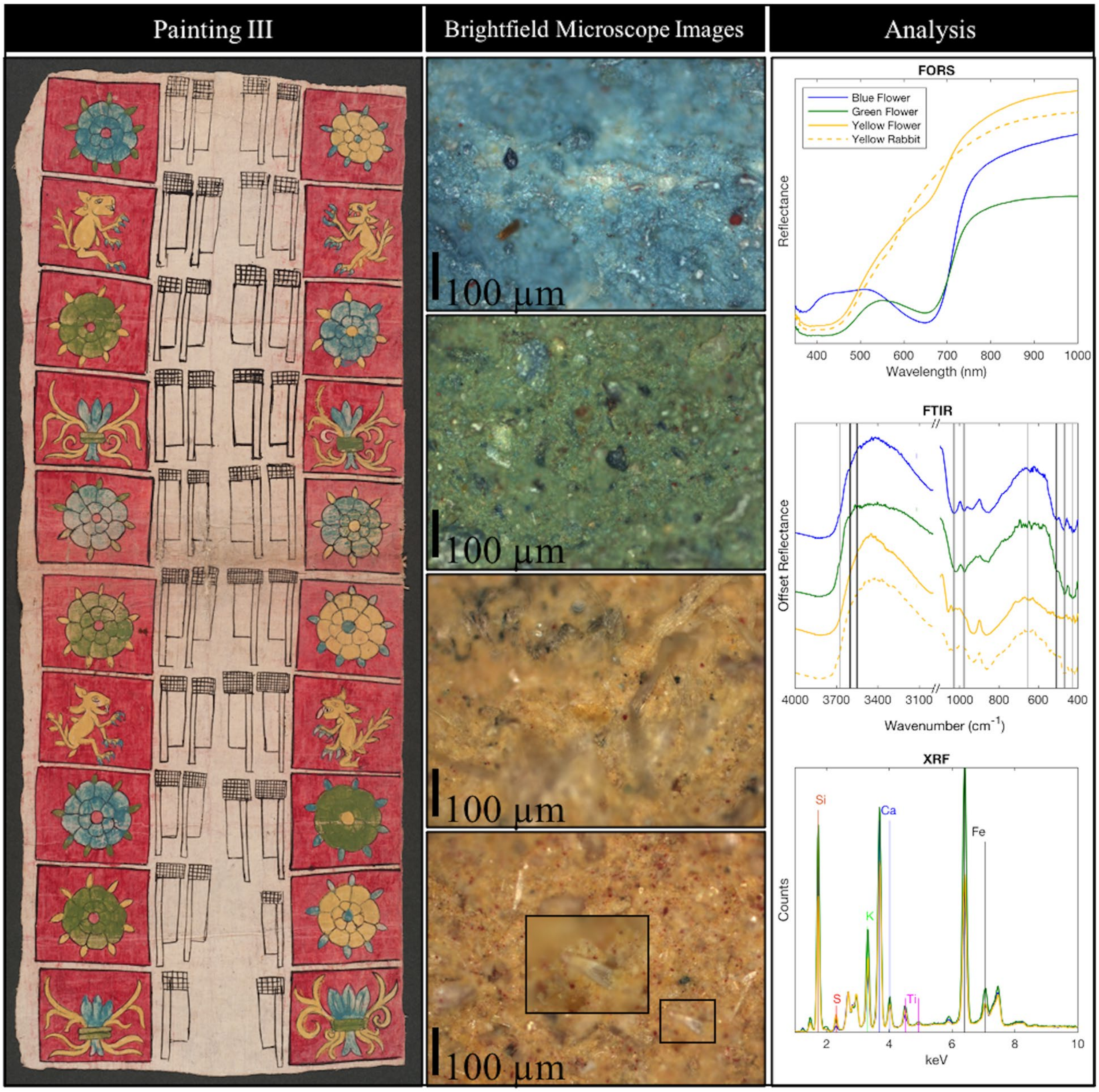

Fig. 7 Digital photograph of Painting III, along with microscope images (taken with a ×20 objective) and respective FORS, ER-FTIR, and XRF analysis from the blue, green, and yellow Flower glyphs and the yellow Rabbit glyph. An intact diatom is highlighted in the inset microscope image from the Rabbit (taken with a ×50 objective). Peaks associated with Maya blue (indigo, sepiolite, palygoskite) and silicates due to diatomaceous earth are marked in the spectra

the findings of a palygorskite and sepiolite mixture are, thus far, unique to only these two codices.

The yellow in the Flower glyphs in Painting III is characterized by a strong and broad absorbance between 350 and 440 nm and another small absorption feature at 665 nm. According to the thesis of Nabais that took an in-depth look at yellow colorants used in Mesoamerican codices [44], these spectral features could indicate the use of *zempoalxóchitl*, an organic dye extracted from the yellow or orange marigold flower (*Tagetes erecta*) that is native to southern Mexico. This would be a unique finding as the most cited organic dyes used for yellows in Mexican codices [4, 24], and those discussed in the Florentine Codex [1], are from the plant sources *zacatlazcalli* (*Cuscuta tinctoria*) and *xochipalli* (*Cosmos sulphureus*). Unfortunately, the yellow pigment cannot be

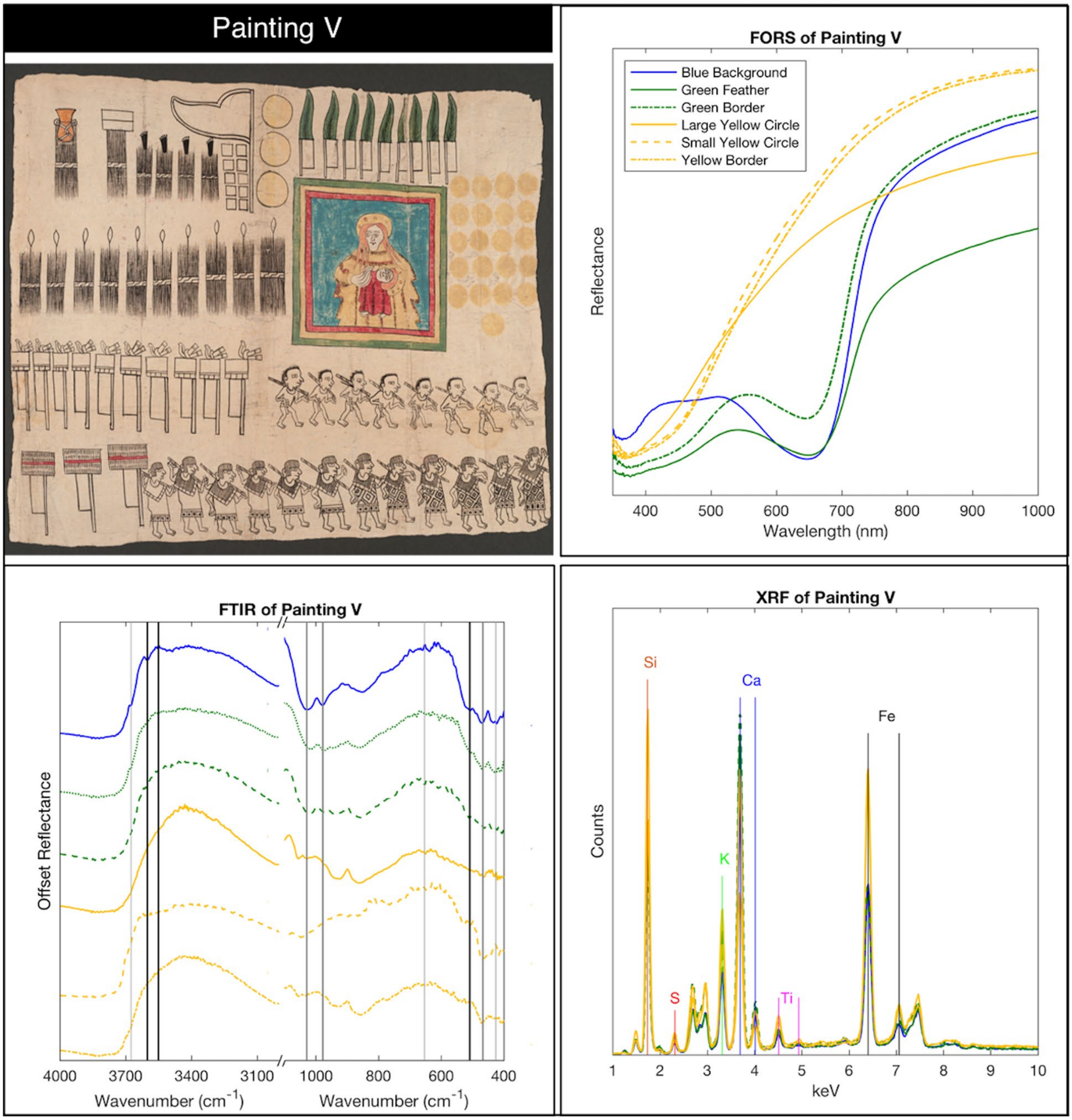

Fig. 8 Digital photograph of Painting V, along with FORS, ER-FTIR, and XRF analysis of blue, yellow, and green areas. Peaks associated with Maya blue (indigo, sepiolite, palygoskite) and silicates due to diatomaceous earth are marked in the spectra

definitively identified (using FORS alone) given the weak reflectance properties in both the visible and infrared range, as well as spectral similarities that exist between many organic yellows in this wavelength range. Yellow painted areas show signals for amorphous silicon dioxide (small feature at 1058 cm^{-1} [45]) in ER-FTIR and Si in XRF. Microscopic investigation discovered the source of these signals to be due to the presence of diatoms. Conservator Albro first detected the presence of diatoms in the yellow pigment samples using PLM (personal communication). An image of an entire intact diatom is seen in Fig. 7, taken from a Rabbit calendrical glyph in Painting III. It can also be seen in the image that the Rabbit is painted from a mixture of the unknown yellow pigment with red particles (identified by FORS as the same alum-based cochineal lake used throughout the paintings). The use of cochineal, an insect-based colorant, in the Rabbit glyphs and its absence in the yellow Flower glyphs may be indicative of how the Nahua used specific pigments to infer meanings to images represented [36].

Diatomaceous earth is formed by the fossilized remains of siliceous aquatic algae; this material was called *tizatl* by the Nahua ("white dirt") and was abundant in the basin of Mexico [1, 24]. Although *tizatl* was available in painters workshops, diatoms are not frequently cited as pigments used for painting manuscripts but rather for the ritualistic painting of the skin of sacrifical victims, brave warriors, and women who died in childbirth [3, 46, 47]. To our knowledge, this is the first finding of the use of *tizatl* in combination with a yellow dye. Further, it is probable that *tizatl* may be the material that has yet to be identified in the many findings of an organic–inorganic yellow hybrid pigment [24] often termed "Maya yellow" [1]. This hypothesis will not be possible to confirm without microscopic investigation of other codices, such as codices Cospi [10], Borgia [43], Fejérváry-Mayer [4], Zouche-Nutall, Bodley, and Selden [2] that have yellow areas identified as a yellow-clay hybrid pigment. However, the clay in these yellow hybrids are unidentifiable and termed "clay" as inferred by the detection of silicates. While silicates are often used as markers for clays, Mesoamerican clays used in codex paintings (kaolin, kaolinite, palygorskite, sepiolite) can typically be identified by infrared spectroscopies as discussed in the previously cited research and identified in this study. Therefore, it is feasible that the detection of silicates in an otherwise unidentifiable clay is due to the presence of the siliceous aquatic algae that comprise *tizatl*. Such use of *tizatl* could add new context to the complex symbolism and iconography [1, 3, 5, 46] these ancient Mesoamerican cultures employed when choosing painting materials.

The green areas in Painting III are a mixture of blue and yellow colorants. The green and blue painted areas were found to share several spectral features; a maximum absorbance at 648 nm and the ER-FTIR features of palygorskite and sepiolite (Fig. 6). While there is a possibly that the green areas could be a superposition of indigo and the yellow oxidized formed of indigo (dehydroindigo) [39], the presence of the two clays would seem to indicate that the blue component of the green mixture is likely the same Maya blue used for blue areas alone. It cannot be ruled out, however, that the yellow component of the green mixture is possibly dehydroindigo. The presence of diatoms in the green areas could have bolstered an argument that the same yellow available to the scribes was used with their Maya blue to create the green, but no diatoms were detected in green areas either visually (with a microscope) or spectrally. The inability to detect diatoms does not mean a yellow dye supported on diatomaceous earth can be ruled out; the green paint layer was thick and dense and could have easily hidden small diatoms. Further, the weak signal from amorphous silicone dioxide would be swamped by the silicate signals from palygorskite and sepiolite present in the green paint. This leaves several possibilities for the, ultimately, unidentified yellow component of the green mixture: dehydroindigo, the same yellow used in other yellow-only areas, or any number of other (and numerous) organic yellow dyes available to the scribes.

Painting V underwent the same analysis (Fig. 8) and yielded similar results as Painting III. The FORS, ER-FTIR, and XRF spectral features of the blue and green areas from Painting V are essentially the same as those from Painting III. The blue is a Maya blue with both palygorskite and sepiolite clays. The green appears to share these features, indicating the Maya blue has been mixed with yellow. The yellow on Painting V, however, has no reflectance features in the visible range to distinguish the pigment from the paper support. ER-FTIR and XRF spectra are similar in showing strong signals for silicates and Si, which can be assumed to be due to diatoms as was found in Painting III. While it was not possible to make microscopic visual confirmation of diatoms due to the size of Painting V and stage limitations of the microscope in this current study, diatoms were confirmed during the earlier study by Albro via sampling and PLM.

Discussion

As one of the earliest surviving colonial codices, it is important to place the material findings of this study into context with previous research. These findings are summarized in Table 2, listed with codices that share the same materials. This discussion will exclude the Madrid Codex or other Maya codices; Nahua and Mixtec codices are not only materialistically different from Maya codices, but these cultures are also greatly divided by geography and time.

Table 2 shows that the Huexotzinco paintings and other Nahua codices on amate are all colonial era codices. Pre-colonial codices from similar regions (Codices Borgia, Cospi, Fejérváry-Mayer, and Laud [9–11, 43]) were painted on prepared animal hides plastered with gypsum. While it is known that amate was used for the production of codices in pre-Hispanic times, few remain. One possible reason for their loss is likely due to the increased risk of deterioration as cellulosic materials, as compared to the proteinaceous materials of animal skins. Since the surviving colonial era codices were created on paper, Indigenous and European, this could be an indication of

Table 2 Summary of other codices, their respective regions and time periods, that share the same material findings with the Huexotzinco paintings

Huexotzinco painting materials	Codices sharing similar materials	Codex region	Codex time period
Support			
Gypsum + amate paper	Seldon Roll	Mixtec	Colonial
	Borbonicus	Central Mexico	Colonial
	Azoyu I	Mixtec	Colonial
Blues			
Maya blue (indigo supported with palygorskite + sepiolite)	Borgia	Central Mexico	Pre-colonial
Yellows			
Organic yellow dye with silicates	Borgia	Central Mexico	Pre-colonial
	Cospi (recto)	Central Mexico	Pre-colonial
	Zouche-Nuttall (verso)	Mixtec	Pre-colonial
	Selden Codex	Mixtec	Colonial
	Selden roll	Mixtec	Colonial
	Colombino	Mixtec	Pre-colonial
	Bodley	Mixtec	Pre-colonial
Oranges			
Iron-rich Earth pigment	Vaticanus B	Central Mexico	Colonial repainting
Greens			
Mixture of Maya blue, organic yellow dye, silicates	Cospi (recto)	Central Mexico	Pre-colonial
	Fejérváry-Mayer	Central Mexico	Pre-colonial
	Colombino	Mixtec	Pre-colonial

the change in codex production practices in central and southern Mexico brought about by colonization.

It is worth noting that the codices listed in the table were all on amate paper, whereas four the Huexotzinco Codex paintings are on amate and four on maguey. Since amate was the paper support commonly used throughout Mesoamerica, its use in the Huexotzinco Codex is not surprising. Somewhat surprising is the use of maguey in the same manuscript. However, according to Tirado and Chagoyán maguey paper was produced in Tlaxcala and adjacent areas of neighboring Puebla, such as Huexotzinco, and is likely of pre-Hispanic origin. They suggest that for political reasons the Tlaxcalans made paper from local resources, like agave [48]. While the authors do not extrapolate further, it is possible that the Tlaxcalans and Huexotzincans developed maguey as an alternative to amate because the dominant Mexica exacted large quantities of amate as tribute from their conquered neighbors [49–51]. Therefore, locally-produced maguey paper would be available for use by Indigenous Tlaxcalan and Huexotzincan artists in the production of their own codices.

While Pottier, et al., confirmed that the gypsum found on the Codex Borbonicus was added as a preparatory layer to surface of the amate sheets after they were formed, they also postulate that it might have been added to aid in processing the fibers before pounding into sheets [52]. This hypothesis could point to the use of gypsum as a processing agent in the amate papers of the Huexotzinco Codex since its presence is not visible to the naked eye. Many colonial codices on amate, like the Huextozinco Codex, lack the white preparatory layer as seen on the Codex Borbonicus and pre-colonial codices. Future analysis of other amate codices lacking the preparatory layer would help shed light on whether gypsum continued to be used in Nahua codex production in the colonial era.

The use of carbon black, alum-based cochineal lakes, and Maya blue is common on nearly all codices from central and southern Mexico, both in pre- and colonial time periods [24]. However, as previously discussed, recent research performed on eight pre-colonial and five colonial codices found that Maya blue can have various formulations aside from the "proper" Maya blue of indigo heated with palygorskite clay [12]. The formulation of Maya blue with both palygorskite and sepiolite clay identified on the Huexotzinco Codex has been found in just one other codex, the Codex Borgia, a pre-colonial codex also originating from Puebla. It has been postulated that this formulation in Codex Borgia is due to either a natural occurrence of the two clays mixed together or possibly the result of some experimentation aimed at reducing the amount of palygorskite needed (as it could only be imported from the Yucatan/Mayan areas) [43]. However, the presence of this strange Maya blue formulation on a codex known to be from Puebla, along with a similar formulation on another codex from Puebla, points toward a region-specific way of producing Maya blue. The idea of a unique Nahua method of producing Maya blue in the Puebla region is bolstered by the fact that this type of Maya blue, a formulation using a mixture of both palygorskite and sepiolite clays, is found in two codices that are from different eras, especially considering that most of the pigment findings in the Huexotzinco painting track with pre-colonial painting styles.

Seven codices, a majority Mixtec, all had yellow paint found to be an organic yellow dye supported on an unidentified clay (based on the detection of silicates) [10, 11, 43, 53, 54]. It has been stated that this type of "Maya yellow" has not been found on any other colonial era codex, except for the Selden Codex [43], which, although colonial, was painted in a strictly pre-colonial style and pigment palette. The Huexotzinco paintings are thus unique in being the only other colonial codex featuring a yellow dye supported on a siliceous material (which was identified as *tizatl*, diatomaceous earth).

The rectos of Codex Cospi and Codex Borgia are the only central Mexican codices to have this similar formulation of yellow. Further, Codex Borgia and the Huexotzinco paintings also share the unique Maya blue formulation. This indicates that the scribes of the Puebla area experimented with different techniques to make pigments and interacted with their southern neighbors to incorporate Mixtec techniques.

Codex Vaticanus B is a part of the Borgia Group, a group of pre-colonial central Mexican codices [43]. However, there is debate among scholars on both the original region and time period of the Vaticanus B creation because the painting palette and style fall more in line with Mixtec codices from the early colonial era [4]. Orange paint on the Vaticanus B that is thought to be an early colonial repainting was identified as an ocher, which is an iron-rich clay (although the type of clay was not identified). Both the Vaticanus B repainting and the Huexotzinco Codex are from the early colonial period, lending evidence to the idea that codex scribes quickly moved from organic to inorganic materials under colonization. Iron-rich clays and kaolinite were abundant in central and southern Mexico during pre-colonial times, but most pre-colonial codices were painted with predominately organic materials; oranges were typically created using a mixture with cochineal. It is impossible to say exactly why the arrival of the Spanish caused this shift in material use, particularly when other organic dyes, such as cochineal and indigo, continued to be used (as identified in colonial codices). As previously stated, the change in the palettes of Indigenous scribes may have resulted from societal change as well as a shift in the perception and use of books and manuscripts [35, 38].

Among studied Mesoamerican codices spanning different regions and time periods, the creation of green paints seems to be the least uniform and appears to rely more heavily on whatever materials the scribe had on hand instead of any sort of prescribed practice [4, 11, 43]. For example, the Codices Cospi and Fejérváry-Mayer both made use of a "proper" Maya blue for blue colors and the same type of Maya blue was identified mixed with a siliceous yellow dye. Discussion of all the various formulations of greens found in codices is beyond the scope of this paper, but the Huexotzinco paintings do not appear to be unique in mixing the blues and yellows already on hand.

Conclusion

The results of this analytical study provide a glimpse into the working methods of the *tlacuiloque*, where they continued to use certain pre-colonial painting traditions while beginning to experiment with newly introduced European techniques. The paintings were found to consist of predominantly pre-colonial materials with regard to the paper supports and colorants. Consistent with pre-colonial materials and painting practices are the findings of gypsum on Paintings I, IV, and VI, Maya blue and a yellow organic–inorganic hybrid pigment on Paintings III and V, and the use of an alum-based cochineal lake on seven paintings (Painting VI contains no color). However, the lack of a thick white preparatory layer on the paintings as well as the use of orange kaolinite as a pigment are more in line with colonial codex production.

Unique findings include the use of a Maya blue that was created using a mixture of palygorskite and sepiolite (a similar combination found only on the Codex Borgia) and *tizatl* as the substrate in the yellow organic–inorganic hybrid pigments. It was also proposed that the painting material termed "Maya yellow" uses *tizatl* as the dye support due to the detection of silicates in an otherwise unidentifiable clay, as *tizatl* is a siliceous aquatic algae.

This study was successful in that several colorants from Paintings I, II, III, IV, V, VII, and VIII were identified non-invasively. Knowledge gained about these coloring materials adds to the growing body of ongoing research into Mesoamerican codices, and is particularly important for early colonial codices as very few remain. The blending of pre- and colonial artistic conventions, along with the discoveries of a unique Maya blue formulation and the use of *tizatl* as a matrix for a yellow dye enriches the understanding of Mesoamerican codices by adding new information about codex production practices.

Abbreviations
FORS: Fiber optic reflectance spectroscopy; XRF: X-ray fluorescence spectroscopy; ER-FTIR: External reflectance Fourier-transform infrared spectroscopy.

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Authors' contributions
FORS and XRF data was collected and analyzed by TEV. Digital photographs were taken by MEH. TEV and MEH prepared, revised, and approved the manuscript. ER-FTIR data was collected and analyzed by AS. All authors read and approved the final manuscript.

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Declarations

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Preservation Research and Testing Division, Library of Congress, 101 Independence Ave. SE, Washington, DC 20540, USA. [2]Conservation Division, Library of Congress, 101 Independence Ave. SE, Washington, DC 20540, USA.

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