Article: Ultraviolet-Induced Visible Fluorescence and Chemical Analysis as Tools for Examining Featherwork
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1. INTRODUCTION

Feathers are found in cultural heritage collections of tribal arts from the Americas, Africa, and the Pacific as well as in contemporary art, European and American 18th to 21st century fashion, and in taxidermy and ornithology specimens. Undyed feathers are selected culturally and aesthetically for their color-producing mechanisms as well as mnemonic meanings (Tidemann and Gosler 2010; Gleeson et al. 2012; Riedler et al. 2012), and color is a significant factor in species discrimination in scientific-type collections (Pyke and Ehrlich 2010). Contemporary artists are electing to use undyed feathers for the spectacular addition of color and meaning to composite works (see, for example, Coyne 2014).

In considering the conservation of featherwork, recent research conducted jointly by the UCLA/ Getty Program in Archaeological and Ethnographic Conservation and the Getty Conservation Institute illustrates the importance of identifying feather pigmentation systems in the design of a preventive lighting strategy (Riedler et al. 2014). Undyed feathers can fade at dramatically different rates depending on the natural colorant systems present in the feather and the emission spectrum of the light source. This difference
in susceptibility to fading can be as great as a tenfold difference in light dose. Feathers with color derived from the scattering of light through the nanoscale protein structures are known to be more light-stable than feathers with coloration based on biological pigments. Conservators, therefore, need to be able to estimate the most fugitive colorant source in feathers found in collections, allowing for cumulative display lighting recommendations to be developed for different colorant systems. As all feathers are supported by a keratin structure, photochemical damage to that protein support presents risks even if visible coloration is unaffected.

The authors compared the efficacy of different analytical methods for assessing color loss and photochemical change to feather keratin and carotenoids. These comparisons were conducted following a process of accelerated light aging of white turkey feathers with structural color (i.e., color rendered by the scattering of incident light), and brilliant red biopigmented Scarlet ibis feathers that gain their color from carotenoid sources from ingested plants and insects. Analytical techniques utilized appear in table 1.

Table 1. Techniques Used to Evaluate Photochemical Damage

<table>
<thead>
<tr>
<th>Technique</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV-induced Visible Fluorescence</td>
<td>1. Mini Crimescope Alternate Light Source and 365 nm filter, SPEX Forensics, Edison NJ and Nikon D90 camera with a 2e filter</td>
</tr>
<tr>
<td></td>
<td>2. HQRP 365 nm UV 12 LED Flashlight, LEDs: 12, Wavelength: 365–370 nm, Canon G12 camera with a 2e filter</td>
</tr>
<tr>
<td>Fluorescence Spectroscopy</td>
<td>FLS920 spectrometer from Edinburgh Instruments. To minimize scattering caused by feather structure, the monochromatic slits are set at 4 nm.</td>
</tr>
<tr>
<td>Reflectance Spectroscopy</td>
<td>Control Development Model PDA 512 USB/380-900/1/100μm;</td>
</tr>
<tr>
<td>Spectroscopy</td>
<td>Ocean Optics Halogen Light Source HL 2000; 0/45 angle set up with AVANTIS AFH–5 multiangled head.</td>
</tr>
<tr>
<td>Fourier Transform Infrared Spectroscopy</td>
<td>Perkin Elmer Spectrum 100 FTIR spectrometer equipped with a universal attenuated total reflectance (ATR) sampling accessory. Feathers were placed face down on the ATR crystal of the FTIR and force was applied using the pressure arm until acceptable signal to noise was achieved; four scans were averaged for each spectrum</td>
</tr>
<tr>
<td>X-Ray Photoelectron Spectroscopy</td>
<td>AXIS Ultra DLD spectrometer from Kratos Analytical. Feathers were affixed face up to the sample holder using copper tape. The spectra were measured using a monochromatic Ar+ ion source; charge neutralization was employed due to the insulating nature of feather samples. Binding energy was calibrated using the C 1s peaks (BE = 285 eV). Broad band spectra as well as high resolution spectra for the C 1s, N 1s, O 1s, and S 2p regions were collected. It was necessary to average 3 scans for the S 2p region to obtain an adequate signal to noise ratio.</td>
</tr>
<tr>
<td>Gas Chromatography/Mass Spectroscopy</td>
<td>A ZB-1 (30 m × 0.25 mm × 0.25 μm) capillary column was used for the separation. Helium carrier gas was set to a linear velocity of 50 cm · sec⁻¹. Split injection was used 20:1 and was set to 270°C. The MS transfer line was set to 280°C. The GC oven temperature program was 105°C; 40°C · min⁻¹ to 280°C; isothermal for 1 min. Total run time is 5.38 min. SIM conditions were used (m/z 268). For GC/MS the derivitization solution is prepared by mixing 0.5 mL dry pyridine with 1 mL N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) and 60 μL of this solution is added to each vial. Vials are capped and heated for 15 minutes at 75°C, they are then immediately injected into the GC/MS.</td>
</tr>
</tbody>
</table>
A surprising outcome is that UVIVF, a common nondestructive conservation tool rarely used for the examination of featherwork, can aid in the discrimination between certain feather color mechanisms as well as provide evidence of photochemical degradation. We found that shifts detectable under UV-induced fluorescence may provide an indication of past lighting exposure, contributing important information for calculating museum lighting for current and future display.

2. GOALS FOR FEATHER ANALYSIS

Original goals for the UCLA/GCI study were to determine whether both structural and biopigmented feathers were equally sensitive to accelerated light aging, and whether optimum methods for detecting and quantifying photochemical damage to feather pigments and keratin could be developed. Results from the first study have been reported elsewhere (Pearlstein and Keene 2010; Riedler et al. 2014), and results of the second study emphasizing analysis of photochemical damage to pigments and protein are presented here.

The application of protein amino acid analysis as a marker of light-induced degradation has been limited in the field of cultural heritage and requires the removal of a small sample, adding a methodological disincentive. Schilling et al. (1996) and Schilling and Khanjian (1996) applied gas chromatographic methods to amino acid analysis to distinguish between different proteinaceous binding media in paintings. We also looked to the work of Vanden Berghe (2012) and Vanden Berghe and Wouters (2005) who measured ratios of cystine to the oxidation products cysteine and cysteic acid in wool tapestries, finding increased oxidation products to be a reliable marker of photodegradation. These authors used acid hydrolysis and prederivitization, followed by high-performance liquid chromatography (HPLC) to detect amino acid ratios, and found this technique to be a more sensitive marker of oxidation than either color change or strength measurements. The use of amino acid ratios has also been applied to evaluate preservation states in the fibroin of silk textiles, with dry thermal aging and UV radiation altering the alanine to glycine amino acid ratios (Zhang et al. 2011).

We are not aware of previous studies characterizing the amino acid ratios in feather keratin as an indicator of photodegradation. Photo-oxidation of the chemical structure of feather keratin produces breakage of the disulfide bonds in the cystine amino acid, a reaction estimated by other authors to be detectable through either spectroscopic or chromatographic methods. Photo-oxidation of feather keratin has also not been systematically studied, while photolysis of wool keratin has received considerable attention and will be further discussed.

3. ULTRAVIOLET FLUORESCENCE

UVIVF occurs when shorter energy photons of UV wavelength are absorbed by materials that act as fluorophores by reemitting energy at longer visible wavelengths. When observed under visible light in which UV is excluded, fluorophores can also be chromophores and appear colored, or they may appear colorless, such as is the case with keratin. It is known that UV-induced fluorescence can be useful to natural organisms as an energy-quenching mechanism that converts potentially damaging UV radiation to a range of longer wavelengths that are less harmful or even useful for biological functions (Maxwell and Johnson 2000).

3.1 UV FLUORESCENCE OF FEATHER PIGMENTS

A number of feather pigments have been identified as having significant UV-induced fluorescence (Völker 1936, 1937). Völker (1965) summarized UV fluorescence as found within feathers of bird genera as follows: Type 1 *Cacatua*—gold-yellowish fluorescence (specifically the yellow crown feathers of a group of cockatoos and cockatiels) (figs. 1a, 1b); Type 2 *Melopsittacus*—sulfur-yellowish
Fig. 1. (a) Wing and crest feathers of a cockatiel under diffused light ( Courtesy of Ellen Pearlstein); (b) Wing and crest feathers of a cockatiel showing UVIVF of psittacofulvin pigments (Courtesy of Ellen Pearlstein)
fluorescence (specifically the yellow head feathers of a specific budgerigars or parakeets); and Type 3: *Palaeornis*–greenish fluorescence (green wing coverts of specific parrots including blue-fronted amazon or *Amazona aestivia*, and parakeets) (figs. 2a, 2b).

As a group, feather pigments with consistent fluorescence include many psittacofulvins found in yellow pigments, predominantly in birds in Australian species in the psittaciform order (including three
genera mentioned earlier) but also more recently found elsewhere, as well as porphyrins found in rusty brown owl plumage, which are described later. Psittaciforms include culturally significant birds such as parrots, macaws, cockatoos and lorikeets, whose plumage comprises not only yellow feathers but also green feathers colored by mixtures of structural colors and yellow psittacofulvins.

Following the identification by Völker of a fluorescence generated by yellow psittacofulvin pigmented feathers, numerous other authors have reported similar and expanded observations (Boles 1991; Arnold et al. 2002; Hausmann et al. 2003; Pohland 2006). In evaluating study skins in Australian museums, Hausmann et al. (2003) detected red, orange, yellow, and green fluorescence in 68% of surveyed psittaciforms species examined with a UV source with a peak at 365 nm (62). Pohland (2006) in his dissertation detected fluorescence in plumage on study skins from 181 species within 14 families. Barreira et al. (2012) recently brought quantitative methods to biological fluorescence by measuring green fluorescence from green plumage of the blue-winged parrotlet (Forpus Xanthopterygius) with spectrofluorometry, and these authors are the first to report a violet fluorescence restricted to blue feathers, which are estimated as being structurally colored but with a small biopigment concentration.

Utilizing UVIVF, collections stewards might augment visual observation to better estimate both feather sources as well as pigment types. Pohland (2006) concludes that parrots are the taxonomic category most likely to include fluorescent plumage, i.e. more than a third of all parrots exhibit fluorescent plumage, and fluorescence has been found in plumage from every biogeographical region in the world. In an online discussion group devoted to parrot genetics and coloration, breeders remarked about how fluorescence in parrot plumage increases as birds mature, a subject for further study (List n/d). Various authors (Pearn et al. 2001; Parker 2002; Pearn et al. 2003; Pohland 2006) have hypothesized about the biological signaling advantages of fluorescent plumage, postulating also that it is characteristic of birds living in regions with exposure to a great deal of UVA energy, which is absorbed and then dissipated through the mechanism of fluorescence. A connection between past UVA exposure and visible UV fluorescence was determined experimentally in the current study, where UV-induced fluorescence has been confirmed as an indicator of past lighting exposure.

The identification of showy parrot feathers on cultural objects is simpler than the identification of many other feather types, especially through reference to websites that aid in identification (Assen 2011–12). Birds in the Cacatua genus may appear almost pure white or very pale yellow until viewed with UVIVF. It is more difficult to identify plumage sources when only partial or less visually familiar feathers are used or extant. The following example illustrates the cultural value of identifying psittaciform feathers used on a sculpture. Parrot and macaw feathers are not native to the area where the sculpture originates, and can therefore provide information about the selection of exotic species and suggestions about means of procurement.

Feathers estimated to be from blue-and-yellow macaw (Ara ararauna), African grey parrot (Psittacus erithacus) and yellow-naped parrot (Amazona auropalliata) were found on a contemporary Hopi wood carving from the American southwest (Ledoux 2010) (figs. 3, 4).

The presence of a violet fluorescence from red regions and a yellow fluorescence from a central yellow and black feather provide further indication that psittacofulvin pigments are present, supporting the feather attributions. Parrots and macaws are not traditionally native to the American southwest, and their feathers found there have historically been thought of as exotic imports from Mexico (Eckert and Clark 2009). In the case of this contemporary sculpture carved in 2001 by Gregory Lomayesva, many of the feathers used are from endangered or protected species (Federal Register 2009). As these feathers are unavailable for sale, those used on this sculpture may be from domestic birds owned by the artist, or may have been given to the artist through the Feather Distribution Project, a wildlife protection effort that has provided feathers to Native American artists and religious practitioners living in Arizona and New Mexico (Reyman 2007). Confirming the feather types is significant not only to preventive conservation practice, but also for the cultural information it provides.
Fig. 3. Mask, Gregory Lomayesva, 2001, wood, paint, feathers, 45 cm (l); 27 cm (h); 16 cm (d) (Courtesy of Zena Pearlstone, photo by Nicole Ledoux)

Fig. 4. UVIVF image of feathers on sculpture by Gregory Lomayesva shown in figure 3 (Courtesy of Nicole Ledoux)
Another culturally significant feather pigment for which fluorescence is an identification aid is porphyrin. Porphyrin is a reddish-brown pigment displaying a pinkish purple fluorescence, and is found in 13 different orders of birds (McGraw 2006). Porphyrin-containing feathers from owls are of notable cultural importance. Various owl feathers are significant in Native American regalia, including materials drawn from California, the Plains, the Southeast, and extending into Central America (Howard 1957; Gillespie and Joyce 1998; Gleeson et al. 2012). Porphyrin is light sensitive. In an empirical study where owl feather fluorescence was evaluated as a marker of owl feather age, fluorescence of newly molted feathers displayed a strong fluorescence in contrast with a complete lack of fluorescence on year old and permanent plumage on the same individual (Weidensaul et al. 2011). Porphyrin pigmentation is found on feathers that also contain more light-stable melanin pigments. The porphyrin is restricted to the light-protected base of dorsal sides, and to the ventral sides of feathers, concentrated on the proximal third of newly created feathers, on the underwing coverts, and on down feathers, all areas protected from light on the body of the bird (Weidensaul et al. 2011) (Riedler et al. 2014, 54).

As in the case of psittacofulvins, the presence of fluorescence associated with porphyrins can aid collection stewards in identifying plumage of a number of different owls. The well-documented light sensitivity of this pigment and its correspondingly fugitive UV-induced fluorescence, contributes to the evaluation of previous light exposure of these feathers throughout their entire useful life. If fluorescence is found on owl feathers used on regalia, it would suggest that newly molted feathers were used and were protected from light during all stages in the manufacture, use, and storage of the featherwork, as well as following museum collection. We have not detected porphyrin fluorescence on owl feathers found in museum collections.

3.2 ULTRAVIOLET FLUORESCENCE OF KERATIN

UVIVF was recognized as a significant tool for the examination of both wool textiles and feather materials soon after UV lamps became commercially available in 1925 (De la Rie 1982, 2). In a 1927 publication of the *Journal of the Textile Institute Transactions*, British industrial researcher H. R. Hirst described an UV-induced blue-white fluorescence detectable in wool as “characteristic of some of the amino compounds produced by the decomposition of keratine (*sic*). Cystine shows no distinct fluorescence; leucine, asparagine, tyrosine, alanine, and glycocol fluoresce similar to wool” (Hirst 1927, T372). Hirst goes on to acknowledge the likely benefits of exploring UV fluorescence as a way to characterize keratin decomposition products. Although multispectral imaging has been adapted for the detection of fluorescent dyes and pigments on textiles, until recently the application of UV fluorescence to wool fabrics has been limited to the detection of optical brighteners, biological alteration products, and stains (Andrew and Eastop 1994).

Fluorescence characteristics of degraded keratin have been thoroughly explored through the examination of wool and impacts of industrial processes (Collins et al. 1988; Smith 1995; Davidson 1996; Sionkowska and Planekca 2011). Various authors have noted that the cystine disulfide bond essential to the keratin structure quenches UV-induced fluorescence and that treatments that disrupt disulfide bonds may cause energy to be emitted as fluorescence (Collins et al. 1988, 349–51). Collins et al. (1988) noted the development of a blue fluorescence under a 365 nm source following the application of oxidizing agents to wool keratin. Davidson (1996) and Dyer et al. (2006) looked specifically at photo-oxidation and its effects on tryptophan, and on the oxidative coupling of cystine. An important difference between the amino acid makeup of wool, which is α-keratin (helical in structure), and feather, which is β-keratin (pleated sheet structure), is tryptophan, which is a major constituent of wool and is minor or unreported in feathers (Schroeder et al. 1955, 3904; Arai et al. 1983; Murphy et al. 1990, 918).

Davidson identifies tryptophan degradation products as the source of blue fluorescence in wool keratin following irradiation at 360 nm (1996, 7). Sionkowska et al. (2011) found that keratin chromophores...
with fluorescence between 360 and 500 nm developed following 60 min of peak irradiation of wool at 254 nm, and ascribed these to tryptophan deterioration. Researchers examining chemically induced fluorescence and phosphorescence (or chemiluminescence) of four proteins including white feather keratin-detected luminescence of both collagen and keratin samples following irradiation between 320 and 400 nm, concluding that chemiluminescence occurs even in the absence of tryptophan (Millington et al. 2008). While not linked by earlier authors to a fluorescence change, wool keratin has been noted to experience measurable bleaching and fading upon exposure to visible blue light (Lennox and Rowlands 1969, 359).

Keratin hydrosylates from feather protein include the breakdown of cystine to cysteic acid (Sionkowska et al. 2011). Free radicals of sulfur are known to be reactive and destructive to keratin. The effect of irradiation on cystine “involves the initial formation of its monoxide, followed by dioxide formation and then cleavage by water to generate cysteic acid” (Davidson 1996, 5). Cysteic acid, cysteine S-sulfonate, and cysteine thiol groups have been identified as photodegradation products of cystine in all keratins using FTIR and Raman spectroscopy, used to detect photolysis of the disulfide bonds (Millington and Church 1997). The connection between formation of these products and UVIVF has not been suggested for featherwork, and thus provides the basis for the current work.

4. MEASURING PHOTOCHEMICAL DAMAGE IN FEATHERS

Beyond knowing that UV fluorescence is a stable attribute of some feather pigments, and a light-sensitive attribute in others, we were motivated to determine whether color shifts visible in UV-induced fluorescence could provide a nondestructive marker of photochemical change to the keratin feather structure. We detected changes in UVIVF and appearance changes in accelerated light-aged feathers, lending credence to the early remarks of Hirst who suggested that a blue-white fluorescence is indicative of a degradation product within wool keratin. To understand the chemical processes corresponding to observed changes in UV fluorescence in feathers and to quantify fluorescence outcomes, a pilot study was conducted where spectroscopic and chromatographic techniques outlined in table 1 were utilized on aged and unaged samples of study feathers.

4.1 MATERIALS AND METHODS

4.1.1 Feather Samples

Two feather samples were selected for the application of color measurement and analysis before and after accelerated light aging. Feathers utilized in this study are pure white wing feathers lacking biopigment, from the domestic Broad Breasted White Turkey (Meleagris gallopavo), and scarlet ibis (Eudocimus ruber) wing and tail feathers with brilliant red carotenoid pigmentation (Fox 1962). The distributor of the commercially purchased turkey feathers confirmed that they are washed with a detergent called Wool Wash, which contains triethanolamine, sodium lauryl sulfate, various surfactants, mild alkalis and glycerin, and are then sterilized with hydrogen peroxide. These feathers were selected because they are pure keratin that reflects all visible wavelengths equally.

The scarlet ibis feathers were collected from four different zoos, each committed to maintaining plumage color to breed standards through a carotene rich diet. These feathers were selected because previous research provided information about the light sensitivity of the carotenoid biopigments present (Pearlstein and Keene 2010; Riedler et al. 2014). Scarlet ibis feathers were observed to display
considerable variation in color between feathers and within single feathers. As determined through pigment extraction carried out by McGraw, pigment content varied from 47 to 938 mg of pigment per gram of feather on unexposed feathers. The largest intra feather variation was a difference of 417 mg between the feather tip and the lower portion, with the tip having the most pigment and appearing most saturated.

4.1.2 Accelerated Light Aging

Feathers were exposed to four lighting environments to represent accelerated examples of conditions present in the illumination of plumage on the bird, on items during cultural use, and within the parameters of museum display (table 2).

In each instance the feathers were enclosed in semiopaque card stock enclosures that uniformly reduced light exposure on the bottom halves of each feather. Control feathers were stored in a dark closet at otherwise ambient conditions. The feathers were exposed in all four environments for time periods of 3, 10, 29, and 100 days. Total exposure hours as well as emission spectra vary under different aging conditions as both natural and artificial sources were used.

5. ASSESSMENT METHODS AND RESULTS

5.1 ULTRAVIOLET-INDUCED VISIBLE FLUORESCENCE

Pure white feathers selected to display the impact of light on unpigmented feather keratin demonstrate fluorescence changes following accelerated aging. Exposure-induced change in keratin fluorescence appears wavelength dependent, and specifically responsive to the presence or absence of UV in the spectral power of the light source. Unexposed feather shafts produce a characteristic white
appearance under UV, while the unexposed barbs and barbules (side branches and hooks creating the vane) are a lower density of keratin and exhibit a pale blue fluorescence. White feathers exposed to UV-filtered light do not exhibit color shifts even after 100 days of accelerated aging when observed under visible light; however, shifts in color are detectable with UVIVF. UV-induced fluorescence was revealed to be a sensitive technique for assessing light-induced degradation in turkey feathers, as changes in appearance were clearly detectable after only three days of aging independent of the type of aging; however, the consequential changes in UVIVF were different depending on the aging conditions to which the feathers were exposed.

After three days of UV-filtered museum lighting, exposed regions of white feathers observed under UV display a darkening and a shift toward blue-violet, while the unexposed region remains unchanged (fig. 5).

Window-aged feathers also display an increased darkening and a shift toward blue-violet after 3 days, and feathers aged under extreme UVA conditions exhibit a shift toward yellow despite the absence of measurable colorimetric changes using reflectance spectroscopy. White feathers aged for both 10 and 29 days under accelerated museum lighting conditions continue to further darken and shift toward blue-violet in exposed regions, whereas extreme UVA conditions result in further yellowing in exposed regions, along with the development of fluorophores producing yellow UV fluorescence. The penetration of UVA radiation through the paper mask used to protect the lower “unexposed” regions resulted in shifts of the lower regions toward blue-violet in 29 and 100 days UV-aged feathers (figs. 6, 7).
Fig. 6. UVIVF images of turkey feathers after 29 days of accelerated UVA aging (Courtesy of Ellen Pearlstein)

Fig. 7. UVIVF images of turkey feathers after 100 days of accelerated UVA aging (Courtesy of Ellen Pearlstein)
This blue-violet shift is more extreme but otherwise similar to that evidenced by feathers exposed to window light. After 100 days of aging, exposed regions of UVA-aged feathers have suffered severe visible yellowing and embrittlement and losses to barbs, and the corresponding areas have an intense yellow-white fluorescence.

Scarlet ibis feathers pigmented with carotenoid also exhibit fluorescence change, but are more difficult to assess due to a greater variety of coloration both within and between feather samples. The authors now recognize the limitations of exposing the top half of the feathers for comparison with the lower halves, as color in unexposed samples is often more intense on the upper half of the feather (fig. 8).

When observed under UV light, unaged scarlet ibis feathers also present great variety of appearance: some of the feathers fluoresce emitting in the red part of the visible spectrum, while others do not. Despite this variation, scarlet ibis feathers display an increased darkening and shift toward blue violet under UV with increased exposure to museum lighting beginning with 3 days, or a total of 1.5 Mlux hours. Visible color loss and a corresponding yellow fluorescence occur after 10 days of UVA aging (fig. 9), while other lighting conditions including museum lighting and window lighting result in increased darkening and blue violet appearance in exposed regions.

After 29 days, increased blue violet appearance is evident in UV-filtered (museum lighting) feathers and more strongly in feathers exposed to partially UV-filtered window light; UVA exposed feathers display severe color loss and yellowing in visible light (fig. 10).

As in the case of turkey feathers, scarlet ibis feathers following 100-day aging display darkening and blue violet appearance in regions exposed to UV-free or partially UV-filtered light, and after
Fig. 9. Scarlet ibis feathers after 10 days of accelerated UVA aging (Courtesy of Ellen Pearlstein)

Fig. 10. Scarlet ibis feathers after 29 days of accelerated UVA aging (Courtesy of Ellen Pearlstein)
UVA aging display visible color loss, feather embrittlement, severe yellowing, and yellow fluorescence (figs. 11–13).

The interpretation of color changes detectable by UVIVF have been limited to measurable changes in pigment concentration in the case of scarlet ibis feathers, and to ratios of intact disulfide bonds, i.e., cystine, to oxidized sulfur in the form of cysteic acid or a precursor, measurable in feather keratin by techniques applied below. Previous work has indicated that UV exposure is responsible for destruction of carotenoid pigmentation in feathers (Pearlstein and Keene 2010), and current work indicates further that a reduction in pigment concentration results in a detectable shift in UVIVF. As wool studies indicate the development of new fluorophores and quenching of others in keratin following exposure to specific wavelengths of UV, the same may be occurring in the feather keratin. Precise mechanisms contributing to feather appearance changes induced by UV-filtered lighting are part of ongoing research.

5.2 FLUORESCENCE SPECTROSCOPY

Fluorescence spectra of the feather samples were collected in an attempt to corroborate the changes in UVIVF. The fluorescence changes that are quite noticeable in photographs produce only subtle spectral changes, except in the case of the UV-aged feathers. With an excitation wavelength of 365 nm—comparable to the UVIVF—the emission spectra of turkey feathers UV-aged for 3, 10, and 29 days display a change

Fig. 11. Scarlet ibis feathers after 100 days of accelerated museum lighting aging (Courtesy of Ellen Pearlstein)
Fig. 12. Scarlet ibis feathers after 100 days of accelerated window aging (Courtesy of Ellen Pearlstein)

Fig. 13. Scarlet ibis feathers after 100 days of accelerated UVA aging (Courtesy of Ellen Pearlstein)
with increased aging. Figure 14 shows the emission spectrum of a turkey feather that has been UVA-aged for 0–29 days; the excitation wavelength is 365 nm.

There are numerous sharp peaks present that correspond to structural scattering rather than absorption emissions. There is a slight spectral red-shift, and an average of the change in emissions is centered at 505 nm. This corresponds to an increase in greenish emission, which is observable in the UV-induced fluorescence photographs. It was not possible to gather spectral data for the feather that had been aged 100 days due to extensive feather deterioration. These spectral changes provide data in support of the reduced blue and increased yellowish appearance of UVA-aged turkey feathers.

5.3 REFLECTANCE SPECTROSCOPY

All turkey and scarlet ibis feathers utilized in the study were measured with reflectance spectroscopy both before and after accelerated light aging. Measurements were taken with illumination and detection at two different angles relative to the barbs, and these measurements were recorded separately as P (parallel) and D (diagonal) to the barbs. This method accounts for the anisotropic light reflection of feather structures, and is described elsewhere (Riedler et al. 2014). Mylar templates were created for each feather to assure measurement repetition in the same four locations pre- and post- aging, and two to four measurements per location and per angle were captured and averaged. Differences in color between exposed and unexposed sites were calculated according to International Center of Illumination standards, abbreviated as CIE ‘76 Delta E.

Despite efforts to achieve reproducible color measurement, features such as the camber of individual turkey flight feathers (Scott and McFarland 2010, 51) and minor structural damage in reference scarlet ibis feathers that prevented identical repositioning under the template created...
measurement challenges. These changes also found in control feathers due to the physical repositioning of the feather and template—caused us to be conservative in our assessments of color change. In the case of scarlet ibis feathers, measurable color change was evident in feathers UVA-aged for 10, 29, and 100 days but was only the case for turkey feathers after 100 days of UVA aging. “Unexposed” regions of these turkey feathers also displayed measurable changes as the enclosures were not light tight.

5.4 PIGMENT CONCENTRATION ANALYSIS

Scarlet ibis feather pigment concentration was measured for all control and accelerated light-aged feathers to determine whether a decline in concentration could be correlated with spectral power distribution of the light source, or length of aging. Pigment was extracted thermochemically and analyzed by UV-Vis spectrometry using a method developed by McGraw (McGraw and Nogare 2005) and total carotenoid pigment content was quantified. Separation of the individual pigments was beyond the scope of this study. Maximum spectral value absorptions ranged from 460 to 475 nm, which is consistent with the presence of ketocarotenoids (specifically canthaxanthin). The formula described by McGraw et al. (2003) was used to determine total carotenoid concentration, using the extinction coefficient of 2200 for canthaxanthin. The inconsistency in pigment distribution within unaged feathers makes the interpretation of data difficult. It is not possible to simply record the loss of pigment in aged feathers, since feathers at each point may possess drastically different amounts of pigment. Instead, the pigment content of the tip was subtracted from the pigment content of the covered portion of the feather for each time point to give a “pigment change parameter” (fig. 15).

Fig. 15. Change in pigment concentration versus log time for aged Scarlet ibis feathers (Courtesy of Melissa Hughes)
For control feathers the pigment change parameter is always negative because the tips have more pigment. The window, museum, and UVA-aged feathers exhibit a clear trend of increasing pigment change parameters, indicating that while the feather tips may still contain more pigment than the bases, this difference decreases over time. Only in UVA-aged feathers do the feather tips become less pigmented than the bases. These results are somewhat more sensitive to small changes in pigment content than is visual evaluation, as changes in window and museum-aged feathers could not be observed visually, likely due to the uneven distribution of feather color.

5.5 FOURIER Transform Infrared Spectroscopy

FTIR spectroscopy nondestructively analyzes the vibration of the functional groups present in feather samples. Unaged feather spectra collected in this study were quite similar to previously reported ATR-IR spectra of feathers (Xhang et al. 2012). The spectra of the unaged scarlet ibis and turkey feathers were highly consistent with one another with the exception of a peak at 1040 cm\(^{-1}\), the peak attributed to asymmetric S=O stretching of cysteic acid, which is present in the turkey feathers but absent in the ibis feathers. This apparent species variation is certainly the result of the turkey feather vendor’s disinfecting peroxide wash. In the case of aged feathers, FTIR proved to be insensitive to changes in feather samples aged in window or museum light; however, spectral changes in UVA-aged feathers are pronounced. Most of the spectral changes occur below 1700 cm\(^{-1}\) and are consistent with an increasing concentration of keratin oxidation products. Figure 16 shows the difference between spectra of UVA-aged Scarlet ibis and turkey feathers and their unaged controls; a feather sample that has been chemically oxidized by soaking for 5 days in 30% hydrogen peroxide is also included for reference. The chemically oxidized feather spectra as well as the 10, 29, and 100 day UVA-aged feathers have two clearly defined peaks at 1175 and 1040 cm\(^{-1}\), which can be attributed to the symmetric and asymmetric S=O stretching of cysteic acid. An additional, more difficult to assign band in the feathers is seen at 1090 cm\(^{-1}\). This peak may be attributable to cystine S-monoxide, which typically absorbs at 1070–1080 cm\(^{-1}\). A peak at 1090 cm\(^{-1}\) would be consistent with alanine sulfenic acid that absorbs at 1093 cm\(^{-1}\) (Setiawan et al. 1985).

![Fig. 16. FTIR absorbance differences between spectra of UVA-aged turkey feathers (left) and Scarlet ibis feathers (right) and their unaged controls (Courtesy of Melissa Hughes)](image)

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The changes seen in the spectra of chemically oxidized feather samples are highly consistent with changes in chemically oxidized wool and human hair samples (Shah and Gandhi 1968; Alter and Bit-Alkhas 1969; Strassburger 1985). The cysteic acid and cystine S-monoxide species are both present in UV-oxidized wool. An increase in the absorbance at 1720 cm\(^{-1}\) is observed, appearing as a subtle increase in the shoulder of the prominent carbonyl stretch in the IR spectrum, however in the difference spectrum shown in figure 16, this change is quite prominent. To the best of our knowledge, such a change has not been reported in the IR spectra of oxidized wool or hair; however, a close examination of the published spectra of oxidized wool does reveal an increase in the shoulder of the carbonyl peak (Church and Millington 1996). Because of the density of possible functional groups in this region, this peak is difficult to precisely assign. It is consistent with carboxylic acid or ketone bonds, which could be produced by the oxidation of amino acid side chains or the hydrolysis of the peptide backbone. Alternately, the increase may be due to changes in the keratin secondary structure, as studies of the denaturation and recrystallization of feather keratin have shown a similar increase in this shoulder (Xhang et al. 2012, 23).

5.6 X-RAY PHOTOELECTRON SPECTROSCOPY

XPS monitors electron energy levels and can give information about a sample’s elemental content and bonding. It was applied in this study to determine whether the spectral results would allow for detection of changes in the disulfide bonds. UV-aged feathers show significant changes in their S 2p peaks, indicating increasing presence of oxidized sulfur. In turkey and ibis feathers, the S 2p peak area increases over time. This may be due to cystine residues and their oxidation products becoming increasingly exposed as feather structure breaks down. The broadband XPS spectrum of feathers is quite similar to the spectrum of wool; photoelectron peaks are observed for O 1s, N 1s, C 1s, S 2s, and S 2p at 533, 400, 285, 230, and 165 eV, respectively. In wool, the S 2p region of the XPS spectrum has been extensively studied (Carr et al. 1985; Carr et al. 1986; Carr et al. 1987). In untreated wool, the S 2p peak occurs at 164 eV, corresponding to cystine; wool that has been oxidized by plasma, corona discharge, or chlorine water has an S 2p peak at 168 eV, which has been identified as cysteic acid (Carr et al. 1986). Wool that has been treated with performic acid or hydrogen peroxide has peaks at 165.5 and 167 eV, which correspond to cystine monoxide and alanine sulfinic acid respectively. For unaged feathers, the scarlet ibis and turkey spectra already show significant differences in their S 2p peak: Ibis feathers have a fairly clean cystine peak centered at 163 eV, whereas turkey feathers have two additional peaks at 165.5 and 168 eV, which can be identified as cystine monoxide and alanine sulfinic acid on the basis of the wool studies. As noted earlier, this difference is almost certainly due to the vendor’s pre-treatment of the turkey feathers with hydrogen peroxide.

As with the FTIR analysis, XPS is sensitive only to spectral changes demonstrated by the samples that were aged under UVA. The S 2p region in turkey and in scarlet ibis feathers is significantly altered after only 3 days of UVA exposure: In turkey feathers, the cystine monoxide peak at 165.5 is lost, whereas in the ibis feathers, a new peak at 168 eV begins to grow while the reduced sulfur peak at 163 eV decreases in size. After 10 days of UVA aging, the cysteic acid peak becomes more prominent for both types of feather. By 29 days, the reduced sulfur peak is gone in the turkey feathers, while it takes the full hundred days before this peak is eliminated from the scarlet ibis feather spectrum, possibly suggesting a protective role for the carotenoid pigment. Intriguingly, the total area of the S 2p peak increases as the feathers become more oxidized.

5.7 GAS CHROMATOGRAPHY/MASS SPECTROMETRY

Because the changes that were readily apparent as UVIVF in the window and museum light-aged feathers could not be chemically corroborated by XPS or FTIR, and only UVA-aged changes were corroborated, amino acid analyses was performed using GC/MS. On the basis of the work of previous authors who detected light induced amino acid change (see Vanden Berghe 2012) as well as the XPS and
FTIR results, we focused our analysis on cystine and its photo-oxidation product cysteic acid. Qualitative measurement of amino acid profiles was performed on all pre- and post- accelerated light-aged feathers except those exposed to UVA for 100 days and consequently too damaged, to determine whether fluorescence could be correlated with measurable changes in amino acids.

The amino acids were hydrolyzed by heating in hydrochloric acid under nitrogen and subsequently functionalized with trimethylsilyl groups before injecting into the GC/MS, as reported by (Shahrokhi and Gehrke 1968). Figure 17 shows the change in the cysteic acid to cystine ratio over time. Corroborating the results shown by XPS and FTIR, the cysteic acid content of UVA-aged feathers increases dramatically with time.

It is also intriguing that the cysteic acid to cystine ratio of turkey feathers is almost two orders of magnitude higher than that for scarlet ibis feathers over most of the samples; again, this is probably the legacy of the peroxide wash given to the turkey feathers by the vendor. A trend of an increasing cysteic acid to cystine ratio over time was observed for both UVA and window-aged feathers. After 100 days of window aging, the cysteic acid to cystine ratio is 5 times higher than the average among unaged scarlet ibis feathers and 7 times higher than the average among unaged turkey feathers. For UVA-aged feathers, the results were more dramatic and after only 29 days the cysteic acid to cystine ratio was 26 times higher than unaged ibis feathers and 36 times higher than unaged turkey feathers. Feathers aged under UVA for 100 days suffered disintegration and could not be analyzed.

6. DISCUSSION

6.1 DISCUSSION OF ANALYTICAL RESULTS

A variety of analytical methods were applied to understand the chemical changes caused by different lighting doses on feather keratin with and without carotenoid pigmentation. Color measurement using reflectance spectroscopy presents challenges for measuring the same location and maintaining the feather structure for measurement before and after aging. Analytical methods including FTIR and XPS were found to be capable of detecting dose related molecular changes following exposure of feathers to UVA radiation. In the case of pigment extraction, once results were adjusted to account for variable pigment distribution within each feather, changes in pigment concentrations were reliable.
indicators of increased lighting damage for carotenoid pigmented scarlet ibis feathers. GC/MS found increased ratios of the photo-oxidation product cysteic acid to unhydrolysed cystine, which correlated to increasing exposure to unfiltered UV. The reasons why FTIR, XPS, and GC/MS were not sensitive to changes in feathers aged under museum lighting is being further explored; those techniques may not be sensitive enough to detect light-induced changes from sources without UV.

UVIVF assessment proved to be the most sensitive method for detecting changes associated with light dose, even when no changes were detectable under diffused visible light. Fluorescence changes are evident after a short exposure of three days of UV-filtered museum lighting, as fluorescent behavior changes toward darker blue-violet in white feathers may be correlated with light dose. Inclusion of UVA in the light source produces an accelerated yellowing, detectable as a fluorescence shift in feather keratin compared to blue fluorescence changes of feathers exposed to UV-filtered museum lighting. In the case of carotenoid pigmented feathers, a shift toward less fluorescence and toward blue-violet is detectable, which increases with time under the same light dose. Irradiation with a UVA source produces color loss that is perceptible in scarlet ibis feathers after 10 days under visible light, and fluorescence changes toward bluer or darker, supporting the reduction in biopigments found in the pigment change evaluation. Window aging after 29 days shows further fluorescence changes, and irradiation with a UVA source continues to show both color loss under visible light as well as a shift toward yellow fluorescence.

7. EXAMINING MUSEUM FEATHERWORK

Examining museum featherwork for evidence of fading presents challenges because of the varying sensitivity of biopigments and structural colorants, the complex nature of feather color production, and the typical lack of historical data about lifecycle, cultural, and exhibition use (Pearlstein et al. 2012). Estimating original feather color is difficult because of the pronounced variability within and between feathers of the same species. Dorsal and ventral sides of feathers often differ in coloration, and feather structures permit the passage of light through the vane, which taken together may eliminate options for comparing front and back sides for estimating the impact of lighting on feather coloration (Riedler et al. 2014). Recognition of the value of documenting type and duration of museum lighting as part of a preventive strategy did not develop until the 1970s and 1980s, and such records generally represent only a fraction of the overall exposure, omitting dosages accumulated during cultural use and private display.

Museum featherwork, some with records of estimated display, were examined using UVIVF both to document psittacofulvin-pigmented feathers expected to display fluorescence, and to compare non-psittacofulvin-pigmented feathers with accelerated-aged feather samples. Results of accelerated aging revealed UVIVF to be particularly sensitive to the development of a blue fluorescence on pure white structural feathers, and a purple shift or darkened fluorescence on carotenoid pigmented feathers. Both feather types experienced visible color loss and yellowing upon exposure to UV irradiation, and in these instances color loss was more pronounced under UVIVF. Museum examples were, therefore, examined for the presence of reported fluorescence changes, including a blue fluorescence on more exposed regions, or for color loss and corresponding yellow fluorescence under UVIVF.

An example of psittacofulvin fluorescence was found in a full wing from an orange-winged amazon (Amazona amazonica) in a private collection. Yellow fluorescence corresponds to ventral regions of the feathers where the concentration of yellow pigment is greatest. From the Natural History Museum in Vienna, a rainbow lorikeet (Trichoglossus haematodus) (fig. 18a) INV # 49847 displays yellow fluorescence corresponding to the bright yellow regions of the chest and tail. For comparison, the same bird is shown under visible light in figure 18b.

As both the orange-winged amazon and the rainbow lorikeet examples are drawn from study collections, and are not mounted for taxidermy, dark storage during museum lifespans is estimated.
7.1 EXAMINATION OF FEATHERWORK WITH DOCUMENTED AND UNDOCUMENTED DISPLAY HISTORIES

Feathers with display histories were examined in museum collections including the Los Angeles County Museum of Natural History (LANHM), the British Museum (BM), and the Hibulb Museum and Cultural Center in Tulalip, WA (HCC). An American Indian Hupa/Hoopa Dance cap from California accessioned into the collections of LANHM in 1932, A.2749.32-95 with great horned owl (Bubo virginianus) feathers and red-shafted flicker wing feathers, has been documented as being on display in internally lit cases with unfiltered fluorescent lights in the Native American Hall between 1992 and 2006 (Kajeian 2013) (figs.19, 20).
Fig. 19. Detail of a feathered Hupa/Hoopa Dance cap from California LANHM (A.2749.32-95), ca. 1900s, hide, feathers, cordage, 27 (h) × 60 (l) × 39 cm (w) (Courtesy of Ellen Pearlstein)
Fig. 20. Detail of a feathered Hupa/Hoopa Dance cap from California LANHM (A.2749.32-95) (Courtesy of Ellen Pearlstein)
Not surprisingly, porphyrin fluorescence is not observed on the owl feathers, and the carotenoid pigmented flicker feathers appear more purple than red (fig. 21).

Also at LANHM, a Pomo feathered basket from California was accessioned in 1914 (FA 2458.83-247) and displayed in ca. 1945, again in 1973, and in the Native American Hall from

Fig. 21. UVIVF image of detail of great horned owl (Bubo virginianus) feathers of Hupa/Hoopa Dance cap from California LANHM (A.2749.32-95) in figures 19 and 20, (Courtesy of Ellen Pearlstein)
1992–2006 in internally lit cases with unfiltered fluorescent bulbs (Kajeian 2013). The basket is covered with what are estimated to be red pileated woodpecker scalp feathers, alternating with pale yellow feathers, with dark borders of mallard duck feathers and California quail topknot feathers. Pileated woodpecker feathers are pigmented with carotenoid pigments (McGraw 2006). The red feathers on the three sides of this basket are not visually different under diffused light however under UVIVF one side displays a markedly yellow appearance and the same feathers on other two sides display a reddish purple fluorescence. A similar phenomenon is apparent with red feathers on Pomo basket FA 2458.83-180, which is also documented as having the same 14-year display. A headdress acquired in 1874 by the BM (Am.9064) estimated to be Nez Perce from the Idaho and Montana Plateau has magpie and eagle feathers and what appears to be red- and yellow-shafted flicker feathers on the brow band (fig. 22). Color loss is suspected for these red and yellow feathers based on the contrast with other samples, but color loss appears more pronounced under UVIVF where a pale yellow appearance is visible (fig. 23).

Results of accelerated aging of scarlet ibis feathers suggest that the yellow appearance of feathers may be indicative of greater UV exposure than the same feathers unexposed to UV, which retain a reddish-purple fluorescence.

Featherwork in museums was examined by adjusting overlapping feathers to detect variation in UVIVF, where increased blue fluorescence corresponds to areas of increased exposure. Two examples

Fig. 22. Detail of the brow band and red- and yellow-shafted flicker feathers (\textit{Colaptes auratus cafer} and \textit{Colaptes auratus}) on a Nez Perce headdress, BM (Am.9064) (Courtesy of Ellen Pearlstein)
include white feathers with melanin coloration. An Ojibwe eagle feather fan (1970.09.4) (fig. 24) acquired by the British Museum in 1970 was displayed between 1999 and 2011 under 50 lux, excluding daylight (Davy 2013). A detail image was taken with overlapping eagle feathers moved aside to reveal differences between light protected, fluorescing feathers and blue fluorescing exposed edges (fig. 25). Similar evidence is visible in a war bonnet in the Hibulb Cultural Center made from turkey feathers (identified by the fine parallel striations on the shaft) (Trail 2003), where light exposed edges fluoresce blue while concealed feathers maintain a white fluorescence.
The Ojibwe feathered headdress in figure 26 (AM 1982.Q. 801) was donated in 1893 to the British Museum, with documented display from 1982 to 1986 at "safe levels" without daylight (Davy 2013). Examined for its characteristic complexity, including multiple types of tail feathers that are possibly duck, heron, and falcon on the basis of color, size, and origin, UVIVF illustrates the correlation between purple fluorescing feathers tips and regions likeliest to have received illumination (fig. 27). UVIVF also aids in distinguishing between feathers.
Fig. 27. UVIVF image of Ojibwe feathered headdress in figure 26 (AM 1982.Q. 801) (Courtesy of Ellen Pearlstein)
7.2 NEED TO STANDARDIZE FLUORESCENCE PARAMETERS

The application of UVIVF as an analytical method within conservation that uses a standard and repeatable workflow has only recently been developed (Dyer et al. 2013; McGlinchey Sexton et al. 2014). Fluorescence examination of feathers must be used with caution, as adventitious matter may contribute to fluorescence. Pohland and Mullen (2006) report on a study of the fluorescence of 20,000 museum bird study skins, where they found that museum-applied “preservation agents” contributed to fluorescence in 2% of the examples. The current authors acknowledge the variation produced by fluorescence detection applied to collection items of different size and under different lighting conditions, as well as the variations possible through the use of different cameras, filters, and lamps with different peak wavelengths, spectral densities and angles of emission. Examination of single feathers such as those subject to accelerated aging allows for a more uniform setup than does the examination of complex three-dimensional objects of varying size in a less controlled environment. The current authors suggest that the UVIVF assessment method is most successful when variation can be detected within a single feather, or within groups of related feathers within the same item, and also recommend comparison with feathers aged under controlled conditions such as presented in this article.

8. CONCLUSIONS

The present study demonstrates how examination of featherwork by UV-induced fluorescence may convey information about feather type and past lighting exposure. Observations by bird biologists of UVIVF in yellow, green, and some blue feathers with psittacofulvin pigments, sometimes in combination with structural color, in feathers of culturally significant birds such as parrots, macaws, cockatoos, and lorikeets are introduced to a conservation audience as a diagnostic aid. Fugitive fluorescence of porphyrins found in owl feathers and circumstances necessary to detect this fluorescence are described. UV fluorescence as a marker of photochemical change in feathers is research in progress, but is introduced as a tool for a preventive conservation strategy.

The results of the application of UVIVF assessment as an analytical tool supported by results of destructive chemical analysis of featherwork are reported. Results of analysis indicate that even limited exposure to UV irradiation in the feather’s cultural lifecycle and in museums results in chemical alteration of feather keratin, with long term accelerated exposures producing dramatic damage in the form of yellowing and embrittlement to both turkey and scarlet ibis feathers. Because feathers are molted periodically on the bird, culturally preserved featherwork requires a different strategy for preservation than is found in nature. In the case of turkey and Scarlet ibis feathers exposed to UVA, changes detected in UVIVF may be correlated with chemical photolysis measured using FTIR and GC/MS, and with changes in total pigment concentration in scarlet ibis feathers. Museum lighting with UV filtration also cause changes detectable in UVIVF, suggesting either different photochemical changes, or changes too small to be detected by analytical methods used. Current work includes carrying out additional amino acid and peptide analysis to determine whether fluorophors produced through visible light reactions in wool keratin, i.e., increased cysteic acid and tryptophan, also occur in feathers. Because preventive conservation strategies are often based on a total cumulative radiant exposure that is difficult to reconstruct, the authors are proposing the examination of featherwork with UVIVF as an assessment tool. Increased use of this examination strategy will aid in further refinements in fluorescence interpretation.

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**SOURCES OF MATERIALS**

Turkey Feathers  
SmileyMe  
Arapahoe, NC 28510  
www.smileyme.com
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