Mechanisms and Morphology: An Analysis of Structural Coloration in Peacock Feathers

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Abstract. This study delves into the intricate physics behind the structural coloration observed in peacock feathers, aiming to uncover the underlying mechanisms responsible for their vivid pigmentation. Through a rigorous investigation utilizing high-resolution optical and scanning electron microscopy, the study elucidates the microstructural components of feathers, providing insights into the mechanisms governing diffuse reflection at various angles. The findings highlight the significant roles played by thin film interference and photonic crystal structures in producing the iridescent qualities of peafowl plumage. Additionally, the paper explores the impact of macroscopic randomness and structural variation in enabling color visibility from multiple viewing angles. This research advances our understanding of natural structural color phenomena and contributes to laying the groundwork for potential applications in advanced optical materials.

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INTRODUCTION

The beautiful structural colors exhibited throughout nature in places such as butterfly wings, beetle shells, and iridescent minerals, have long captivated the scientific community and artists alike. These hues arise from intricate nanostructures that interact with light, a phenomenon of great interest across scientific disciplines, including biology, material science, physics, and optics. The insights gained from studying structural colors in birds and other beings hold immense promise for a multitude of practical applications. First and foremost, understanding the genetic, structural, and optical mechanisms behind these colors can lead to the development of eco-friendly pigments and dyes, reducing the reliance on harmful chemicals in the textile and cosmetic industries. Additionally, the knowledge garnered from these natural systems can inform the design of advanced optical materials, such as light-manipulating coatings, sensors, and displays, with applications in fields ranging from consumer electronics to aerospace. Furthermore, by unraveling the secrets of these colors, we can inspire biomimetic design principles for enhanced optical and material technologies, contributing to a more sustainable and innovative future.

Most colors that we observe in nature and in the human-created parts of our world originate from pigmentation, the presence of specific molecules that absorb complimentary colors and allowing the other wavelengths to continue to our eyes, which is what we see. Pigments create the same color both in reflection and in transmission, since they simply remove certain wavelengths from the incident light, allowing the remaining light to either reflect off of or travel through the object. Their color is also not altered when immersed in a medium with a different refractive index, as long as this medium does not have a component that damages the pigment chemically. These

colors can often be extracted and dissolved to create pigments and dyes, which is not possible with structural colors. (Dushkina, 2009)

Structural colors are unique in that that they are produced by scattering of light or reflect different wavelengths of light, ie different colors, at different rates and in different ways. These materials can often be distinguished by their iridescent quality, meaning that it appears shiny and the specific colors seen vary based on the angle of the light hitting it as well as the viewing angle. Another characteristic of structural colors is that they will disappear after some physical alteration or harm to the object. For example, the blue feathers of the macaw will turn gray-brown when smashed between two plates or hammered gently. Perhaps the easiest way to confirm that something must have structural color is observing that it's color changes or disappears when viewed in transmission, ie. with the light source behind the object in question (Bancroft, 1919).

These effects are due to a variety of optical effects, split into the following five phenomena outlined by Kinoshita and Yoshioka in 2005: thin-film interference, multilayer interference, diffraction grating effects, photonic crystals, and light scattering. The first four of these are collectively referred to as "Bragg phenomenon exhibited by structures with periodic morphology," by Dushkina and Lakhtakia, further broken down into effects due to diffraction gratings and periodic layers (thin-film, multilayer, and photonic crystals). The relevant structures in peacock feathers are of this final group, with some debate as to the specific details of the most appropriate model.

The structural color of peacock feathers is created by the organization of layers of melanin granules just beneath the surface. These granules are arranged in an array that may seem to imply some diffraction grating effects, but their period is far too small for this effect. Yoshioka and Kinoshita (2002) showed that since the rod spacing of 140 nm is significantly smaller than the wavelengths of visible light and since there are usually many of these rods, the scattering angle from these rods is very suppressed at wide angles, resulting in each layer of rods functioning as a single layer component of a multilayer interference system.

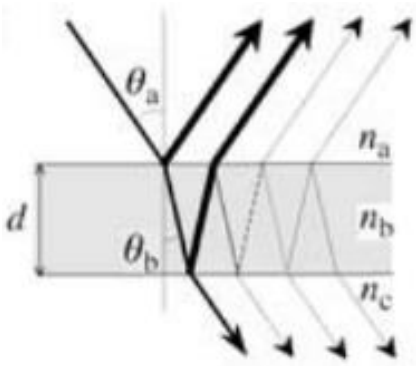

Figure 1. Depiction of thin film interference borrowed from Kinoshita and Yoshioka (2005)

A basic understanding of thin film interference is useful here, as well as a generalization to at least a simple one-dimensional photonic crystal (multilayer interference). Kinoshita and Yoshioka (2005) included a helpful review of these mechanisms. The relevant components are an incident light source, a thin film of thickness d, and a bulk medium. The refractive indices n_a, n_b, n_c in Figure 1 refer to those of air, the thin film, and the bulk medium respectively. Each of the two interfaces created here acts as a partially reflecting mirror, allowing some light through and specularly (in the assumed simple case) reflecting some of it. The interference happens as a result of the difference in path length from light that reflects immediately off of the first interface with light that reflected only off of the second interface. The refractive indices, along with the angle of incidence θ_a can be used to predict the wavelengths λ at which constructive interference will occur:

$$2n_b d \cos\theta_b = m\lambda \qquad (1)$$

This doesn't show a direct relationship with θ_a, but that can be acquired by inserting $\theta_b = \sin^{-1}(\frac{\sin(\theta_a)\, n_a}{n_b})$ from Snell's law. After dividing by m, the reflected wavelength is

$$\lambda = \frac{2n_b d}{m}\cos[\sin^{-1}(\frac{\sin(\theta_a)\, n_a}{n_b})] \quad (2)$$

This relation gives us an expression that can easily be evaluated for wavelengths λ that will undergo constructive interference and therefore reflect well off of this thin film, based on known refractive indices, thickness d of the film, and arbitrary natural number(for $n_c > n_b$) or half natural number(for $n_b > n_c$) values of m. It is apparent from equation (1) and Figure 1 that the wavelengths reflected off of this surface are angularly dependent, which is an essential feature of many structural colors, namely iridescence.

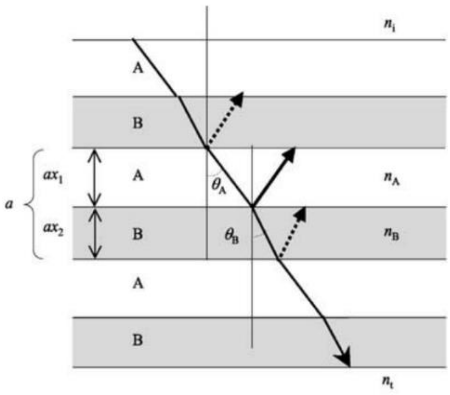

Figure 2. Depiction of a multilayer interference stack borrowed from Kinoshita(2005)

When this effect is generalized to many such layers stacked on top of each other, the mechanism is known as multilayer interference. The two materials will now be referred to as A and B, with refractive indices n_A, n_B and thicknesses d_A and d_B respectively. Also assume $n_A > n_B$. Taking θ_A and θ_B as the respective angles of refraction, the following generalization of equation (1) can be derived:

$$2(n_A d_A \cos\theta_A + n_B d_B \cos\theta_B) = m\lambda \quad (3)$$

Since A is the first layer in this model and has the higher refractive index, the highest level of constructive interference is achieved when λ also satisfies the following relation, for $m > m'$:

$$2n_A\, d_A \cos\theta_A = (m' + 1/2)\lambda \quad (4)$$

These equations describe the infinite ideal multilayer, which is the simplest case of a 1D photonic crystal.

As discussed above, there is still some debate regarding the precise structures that create each component of the brilliant colors of the peacock feather eye. Until recently it was assumed that it was entirely due to the photonic crystal-like structures formed by the arrays of melanin granules, either a similar structure to the multilayer interference described above (Yoshioka and Kinoshita, 2002), or a slightly more complex variation (Zi et al., 2003). An addition to this theory is an analysis of the contribution of thin film interference for certain wavelengths in the peacock feather color. Here, Okazaki's work is particularly instructive, both toward understanding the mechanisms and experimental design. Circularly polarized light(CPL) changes handedness upon reflection, so analyzing the spectra and polarization of light after reflection on a thin film provides a method of distinguishing between thin film interference as the source of the structural color as opposed to other sources that result in diffuse reflection. These other sources include the spongy layers present in parakeets as well as the photonic crystal structure of the melanin granules in peacock feathers. This type of analysis allows a confirmation that a sample does in fact have structural color due to thin film interference, and in the case of the peacock feather, even a distinction showing which specific colors are due to thin film interference, and which come from other sources. (Okazaki 2002)

In this same paper, Okazaki found that the bright color in mallard and rock dove feathers is almost entirely removed when illuminated with CPL and viewed through an opposite-handed cut filter, while the bright colors in parakeet feathers are unaffected by this treatment. This shows that the color in mallard and rock dove feathers comes from thin film interference, while that of the parakeets is from another source. These visual observations are supported by quantitative spectrum data both with and without the cut filter. These spectra showed the disappearance of significant peaks corresponding to the colors that were generated by thin film interference. In the peacock

feather, the blue color in the eye mostly remains(although it is shifted down in wavelength slightly), meaning it is due to the expected photonic crystal effect of the melanin granules. The new evidence discovered here, however is that the green color around the eye disappears when the cut filter was applied, suggesting that much of the color in peacock feathers comes not from diffraction in photonic crystal-like structures, but from thin film interference.

The thin films themselves are composed of the space between the melanin granules and the cortical surface. In the Okazaki paper, TEM imaging was used to observe these structures and determine dimensions for this layer and the melanin granules themselves. The thickness of this layer was analyzed for each type of feather and models were produced to represent the expected reflection spectra due to the thin film. These models roughly corresponded to the observed spectra in each bird, providing further evidence for thin films as a primary mechanism in their production of structural color.

METHODS

Since the structures that create these effects are on the micrometer to nanometer scale, high resolution optical and scanning electron microscopic (SEM) imaging are required to determine their scale and shape. Optical imaging was done using a Nikon Polarizing Microscope owned by the Millersville Physics department, and SEM imaging was done with the help of technician Calvin Montgomery on Millersville University's in-house Hitachi SU3900 Scanning Electron Microscope. The wavelengths emitted are also of interest, specifically the comparison between wavelengths on different areas of a wing or between samples, as well as variation in the color at different angles of incidence. This analysis required usage of the Physics department's Ocean Optics Fiber Spectrometer.

RESULTS AND DISCUSSION

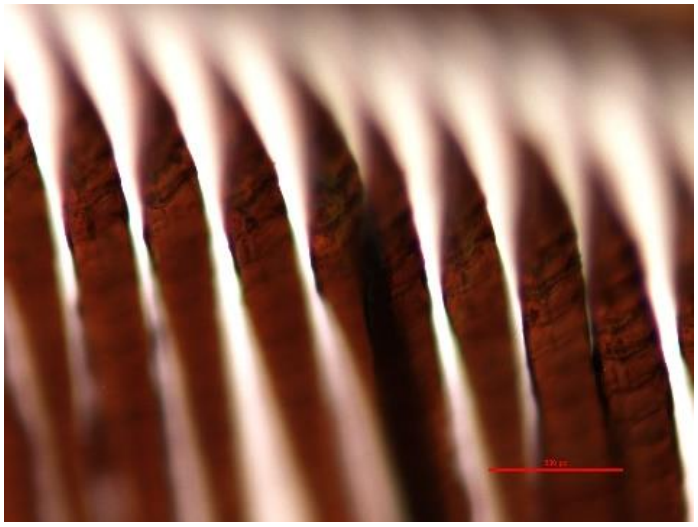

Figure 3. Optical microscopy image of peacock feather barbules from the eye of the feather viewed in transmission(diascopic illumination) under 20x magnification

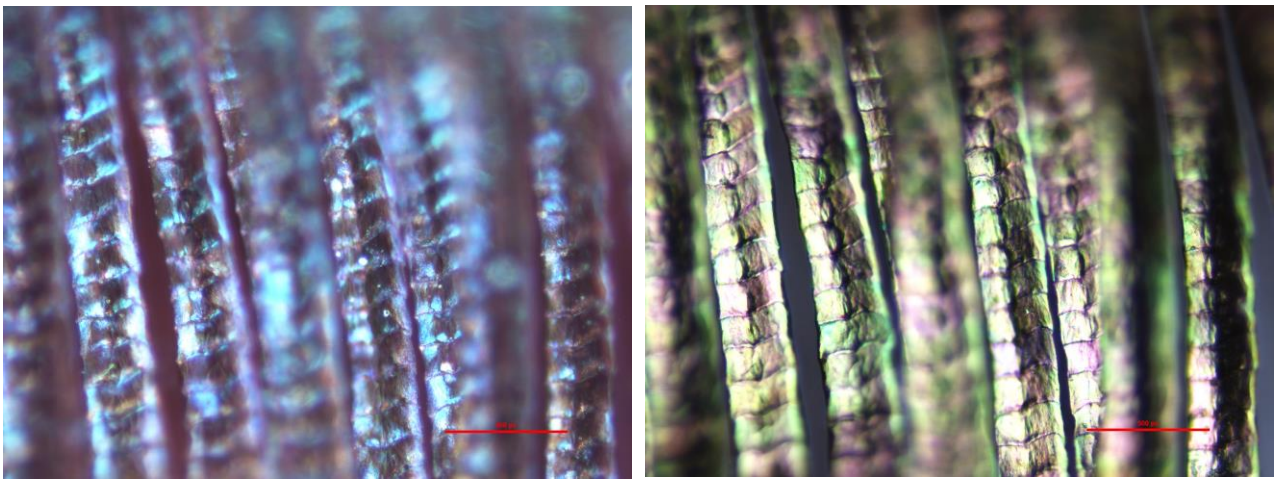

Figure 4. Optical microscopy images of the same location on a strand of peacock feather under crossed (left) and aligned (right) polarizers (both taken with 20x objective, in bright field, under episcopic illumination (in reflection))

Optical microscopy analysis provides evidence that the iridescent colors in the eye of the peacock feathers are in fact the result of structural color. Figure 3 is an optical image with a 20x objective lens and with lighting from the back rather than the top, so the color observed is the result of absorption by melanin granules rather than any physical structure. This gives us the expected brown that melanin allows through. Figure 4 shows 2 similar optical images of a similar area of the eye as figure 3, but now with lighting from the top, so the colors that we see

are now due to reflection. This provides twofold evidence for the existence of structural color here. First, the presence of any significant color at all where there was none in transmission shows that this color must originate from somewhere other than pigment alone. Second, the difference in color between these images shows that the incident light is linearly polarized upon reflection on the surface of the feather. These images are obtained with the polarizing mode of the microscope, with the polarizer and analyzer at 90 degrees to each other on the left, and aligned on the right. The fact that any color is seen at all on the left implies that there must be some linearly polarizing effect by the feather, otherwise all of the polarized light would be blocked by the analyzer (second polarizing filter), and the image would appear black.

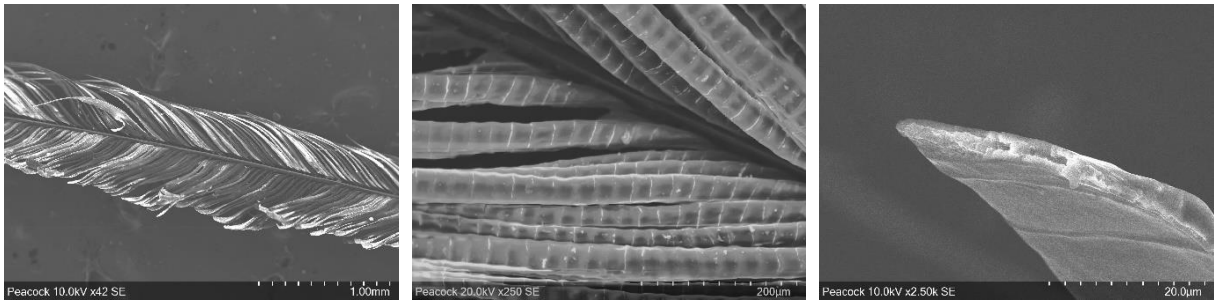

Figure 5. SEM images at various magnifications of representative barbs from a feather of *Pavo cristatus*. The image on the left depicts a portion of a barb, the middle shows many barbules, and the image on the right shows the cross sectional shape of a single barbule. The scale bar represents 1 mm for the left image, 200 µm for the middle, and 20 µm for the right.

TABLE 1. SEM measurements of node dimensions in different regions of the feather. The blue and gold measurements are taken from the same barb taken from the eye of the feather, while the purple sample comes from a large barb on the main stem of the feather. Range is one standard deviation

Area	Node Length (µm)	Barbule Width (µm)
Deep Blue in Eye	25.2 ± 3.26	34.5 ± 5.33
Gold in Eye	29.4 ± 3.22	46.7 ± 2.25
Main Stem	28.6 ± 1.62	60.3 ± 3.46

Scanning electron microscopy allows a more detailed analysis of the specific structures in question. This analysis provides further additions to the understanding of mechanism of peacock structural color require an analysis of the macrostructure of the peacock feather. The problem addressed here is that the mechanisms described in the introduction, regardless of the specifics, explain generation of the color due to ordered lattice of melanin granules, but fail to explain why similar colors are visible from a wide variety of angles. Unlike in morpho butterflies (Kinoshita, S., Yoshioka, S. And Kawagoe, K. 2002) the diffuse nature of the resulting light is caused by the macroscopic structure. As can be seen in Figure 5, the feather is composed of many long thin barbs with length on the order of centimeters, which are in turn made up of many short hairlike barbules, with length of roughly a millimeter. These barbules contain a series of nodes, 30-60 μm wide and 20-30 μm long. These dimensions vary by location as observed in Table 1, however there is no proposed impact of this variation. Importantly, in the cross sectional rightmost image of figure 5, a curve in the shape of the nodes can be observed, creating significant variation in the angle of the granule lattice within them. This creates significant variation of spectra of emitted light throughout a barbule and even between barbules. This reflects what Kinoshita and Yoshioka (2002) observed and through further analysis determined that the statistical sum of these individual areas allows the barb viewed as a whole to have the consistent spectra expected based on the color of that individual barb. Thus, at the submicron scale, the reflection is specular, but when the barbules are considered as a full system, the variation in angle of the granule lattice causes the overall effect is diffuse reflection of a more consistent color than would be expected when simply considering the submicron interference effects.

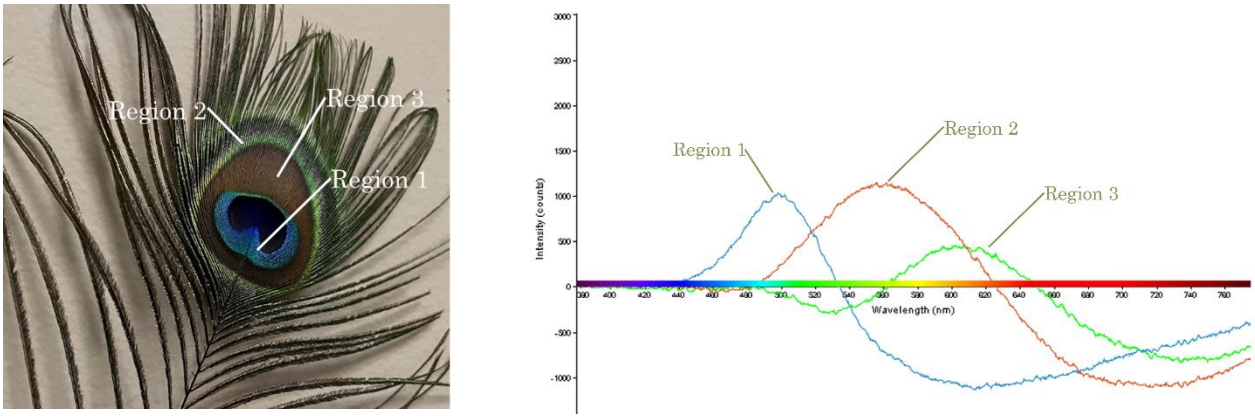

Figure 6. Left image shows 3 regions on the eye of various colors. Image on the right is a visible light reflectance spectrum at each region in arbitrary units relative to a constant incandescent light source. All three were observed at a 45 degree angle of incidence while the image on the left is at roughly normal incidence

Spectroscopic analysis of each region was done with an in house Ocean Optics fiber spectrometer, revealing the expected peak wavelengths varying with both region (Figure 6) and angle (Figure 7). The positional variation provides qualitative confirmation of the observed colors at each location, while the angular data allows us to calculate a theoretical cortical thickness, that is the thickness of thin keratin layer above the melanosomes. This top layer is known to be keratin as assumed by Okazaki, and its refractive index was experimentally determined to be between 1.55-1.6 (Mason, 1923). Taking the refractive index of air to be 1, equation (2) gives the following:

$$\lambda = \frac{2n_b d}{m}\cos[\sin^{-1}(\frac{\sin(\theta_a)\, n_a}{n_b})]$$

$$= \frac{2(1.575)d}{m}\cos[\sin^{-1}(\frac{\sin(\theta_a)}{1.575})] \qquad (5)$$

Now when λ is plotted vs $\cos[\sin^{-1}(\frac{\sin(\theta_a)}{1.575})]$, the slope is determined to be 318.68 nm. Since the slope of that graph is equivalent to $\frac{2n_b d}{m}$, the following equations determines possible values for d:

$$\frac{2(1.575)d}{m} = 318.68 \text{ nm}; \qquad m = 1,2,3 \ldots \qquad (6)$$

In the m=1 case, the thickness d is determined to be 104.8 nm, which is within the error bound of Okazaki's experimentally determined 112 nm thickness. While the thin film model may not fully describe the mechanisms for structural color in peacocks it does provide a meaningful result in this case, and therefore seems to be a useful first step in analyzing the source of structural color in the peacock feather.

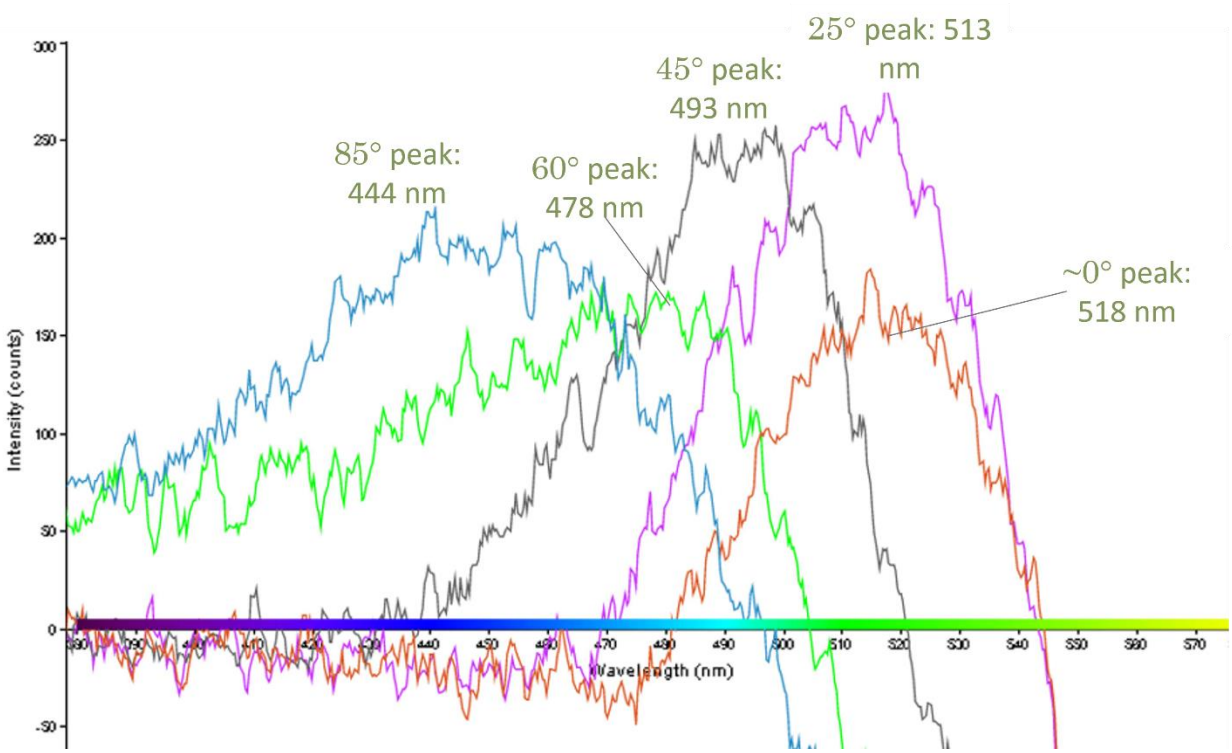

Figure 7. Reflectance spectra of Region 1 at various angles of incidence. The expected trend for thin film interference is observed, where shorter wavelengths are more prevalent at oblique angles and longer wavelengths become dominant as the angle approaches normal. Units are arbitrary and relative to a constant incandescent light source.

CONCLUSION

In conclusion, this study has endeavored to provide a detailed exploration into the complex phenomena underlying structural coloration in avian plumage, with a specific focus on the chromatic attributes exhibited by peacock feathers. Employing an interdisciplinary framework that encompasses biological, optical, and material science perspectives, the research seeks to elucidate the intricate nanostructures contributing to the observed pigmentation. Principal mechanisms such as thin film interference and photonic crystal structures have been identified as influential factors in imparting the iridescent qualities observed in peafowl plumage, along with macroscopic randomness and variation in structure allowing these colors to be seen at many angles. Utilizing advanced imaging techniques, including high-resolution optical and scanning electron microscopy, the investigation aims to reveal the microstructural components of feathers, thereby enabling a nuanced understanding of the mechanisms governing diffuse reflection at different angles. While contributing to our understanding of existing structural color in the natural world and providing an initial foundation for potential biomimetic applications in advanced optical materials, the evolving nature of this field emphasizes the ongoing importance of exploring and refining the proposed concepts.

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