Fourier Blues: Structural Coloration of Biological Tissues

Richard O. Prum and Rodolfo H. Torres

Abstract The non-pigmentary colors of the tissues of living organisms are produced by the physical interaction of light with nanostructures in the tissues. Contrary to what has been previously assumed for many decades, it has been established now that many of the beautiful blue and green colors observed in the tissues of mammals, birds, and butterflies are the result of coherent scattering or constructive interference. Using Fourier analysis one can show that many structurally colored tissues are *quasi-ordered* on the appropriate nanoscale to produce the observed colors by constructive interference. Understanding the mechanisms of coloration in animals is very important because of the role that bright colors play in communication, courtship display, and mate selection in many species of the animal kingdom. In this note we give an exposition of some of the extensive work done recently on nanomaterials with noncrystalline, local scale order. The focus of this article is, in particular, on a truly fascinating manifestation of Fourier analysis and synthesis in nature, which provides a way to explain coloration phenomena that are of interest in behavioral and evolutionary biology.

Keywords Fourier Transform • FFT • Quasi-order • Nano-scale • Nano-structure(s)(d) • Crystallography • Coloration • Scattering • Iridescent • Interference • Electron micrograph(s) • X-ray(s) • Bragg's law • Rayleigh scattering • Benedek

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1 Introduction

The study of the forms of coloration in different materials is a rich, intricate, and multidisciplinary activity. The classic book by Nassau [15] presents a detailed account of at least fifteen different forms of coloration found in our physical world. From a scientific point of view, the explanation of the origin of the colors observed belongs mainly to the métiers of physics and chemistry, but the implications of the presence of coloration in different materials extend to many other disciplines. In particular, coloration as mean of communication plays a crucial role in many areas of biology and the study of species capable of analyzing the complicated color signals. Among such species are certainly humans, and color and coloration play a central role in many situations extending from the scientific, through the practical, to the aesthetic aspects of our lives. Colors allow us to discover and understand physico/chemical phenomena taking place both at microscopic scales invisible to our eyes and at intergalactic distance in our universe; they code, guide, warn, and help us in many aspects of our everyday lives, and they are also capable to stimulate our minds, provoke emotions, and move our souls through the plastic arts.

For biologists it has become clear that the analysis of the mechanisms of coloration, their functions, and evolution can only be studied in an integrative way if one is to fully understand the amazing color displays in many species. In particular, birds are animals capable of communicating though coloration, and the analysis of bird coloration in recent times has refocused some of its efforts to this comprehensive approach. We refer to the two volumes [11] for an extensive account of some of the state of the art in the subject.

Mathematics could not be absent in the explanation of the phenomena of coloration. It is not only present as the universal language of science, but also, through the powerful lenses of Fourier analysis, it provides new explanations and understanding of certain forms of colorations. In this expository note, we will describe some of the developments in which we have been involved in our interdisciplinary collaborations in [19–27]. We will illustrate how Fourier analysis naturally appears in the theoretical formulation of mechanisms of structural coloration through coherent scattering. We will concentrate here on aspects of the coloration of the skin of birds, but the same tools and techniques apply to the study of feathers and other tissues of living organisms.

1.1 Bird Coloration

Both chemical pigments and the physical aspects of the wavelike behavior of light are responsible for the coloration of birds. Pigments have the property of absorbing and emitting selective wavelengths of the ambient light. The resulting colors are determined by the molecular structure of the pigments. Such pigments may be synthesized by the birds themselves or acquired by the birds through their

diet. By removing the pigmentary substance from the tissues the colors disappear, verifying that the pigments are the cause of the coloration. A typical example of pigmentary coloration is provided by flamingos, whose recognizable pink color tends to fade out in captivity through a modification of their diet from the one they have in the wild. Likewise, the black or brown colors of the feathers of a crow or a robin are produced by melanin pigments synthesized by the animal, just as in human black or red hair.

Unlike pigmentary colors (usually yellows, oranges, reds, browns, and blacks) structurally produced colors in avian tissues (often blues and greens) are the result of the physical interaction of light with optical heterogeneities of the tissues. Incoherent Rayleigh scattering has been erroneously assumed to be responsible for the observed non-pigmentary colors of many birds. Rayleigh (or Tyndall) scattering occurs when small, light-scattering objects are randomly distributed without a spatial pattern in the path of the light. Small objects will preferentially scatter smaller wavelengths, giving rise to a bluish or violet color. This mechanism is the explanation for the color of the blue sky. According to this conception of biological structural color, small melanin granules present in the feathers or skin of bird tissues will reflect back short waves, such as violet and blue, but will let pass through longer waves such as red and yellow. The physical and biological literature in the subject can be found in the classical works [10,13,15,35]. A key feature of Rayleigh scattering is that it lacks iridescence or color change with angle of observation, so it was originally applied to all the biological examples of structural color that lack iridescence. Nevertheless, the Rayleigh scattering hypothesis was never supported by spectrophotometric data or microscopic observation of the tissues.

The Rayleigh hypothesis was questioned by Raman [28] in the thirties, but his speculations that color in a certain bird from southern India was produced by constructive interference were dismissed because of the lack of crystalline structure of the bird tissue; see [17]. Dyck [6, 7] in the 1970s was the first to document that the reflectance spectrum of many bird feathers presents a clear peak within the visible spectrum matching the color observed. This is in contradiction with the continuous increase of energy distribution in the direction of the ultraviolet (UV) part of the spectrum that Rayleigh scattering would produce. It was not until the turn of the century that a new explanation for non-iridescent coloration in many animal tissues emerged. The more recent research has established that most greens, blues, and violets observed in birds are in fact structural colors produced by coherent scattering.

1.2 Fourier Analysis Comes in to the Picture

The new explanations about coloration involve Fourier analysis and follow a model by Benedek [1]. The first use of these techniques was our study of the blue feather barbs of a South American bird called the Plum-throated Coating, *Cotinga maynana* (Cotingidae) [22]. The intense blue color of the cotinga is produced by closely

packed spherical air bubbles in the protein of the feathers. This was followed by numerous other works in the study of many other types of structurally colored tissues. The tissues that have been analyzed by now present a big diversity of nanostructures at scales comparable to the wavelengths of visible light. A certain order or periodicity in these structures permits a predictable phase relationship between the light waves scattered and the coherent scattering of certain reinforced specific wavelengths.

Traditionally, the classification of the color-producing structural tissues has been based on the particular physical model used to explain idealized perfectly periodic structures similar to the ones in nature. Nanomaterials may be periodic (or crystalline) in one, two, or three dimensions. These highly periodic materials produce iridescent colors which change in hue with the angle of observation, as typically seen in hummingbirds or peacocks. However, quasi-ordered materials lack periodicity at longer spatial scales, but are still substantially ordered at local spatial scales. There were no traditional physical methods for analysis of constructive interference by materials with only local order, which led to the application of Fourier analysis to the problem.

In our approach we use Fourier analysis to study the geometric nanostructure of 2D transmission electron microscope images of these color-producing tissues. This gives as a frequency content analysis of the images that we use to produce a prediction or modeling of the coherent scattering behavior of the tissue. We also compare these predictions with the reflectance spectrum of the colorful tissues measured with a spectrophotometer. The reflectance spectrum gives the relative intensity of energy at different bandwidths within the visible spectrum.

The use of Fourier analysis in the study of structured materials has a long history. For example, the structure of crystals and quasicrystals can be studied by looking at the diffraction patterns obtained when a crystalline material is illuminated with X-rays. Mathematically, this essentially accounts for the analysis of the Fourier transform of the characteristic function of the crystal or the density of mass function. We refer the reader to the book [29] for a very nice introduction to the subject.

Some of the patterns obtained in crystallography can be explained by *Bragg's law*, named after the only father-son team of Nobel laureates. They were the first to describe the phenomena of X-ray diffraction by crystals [2]. In very ordered materials two parallel incident electromagnetic waves will bounce from scatterers in the material and arrive at a distant observer with a lag in phase produced by the different distances traveled (path addition). This difference in phase produces the reinforcement of certain waves with appropriate wave numbers and the cancelation of others. For this to happen, the wavelengths and the physical distances defining the ordered structures in the material have to be of comparable size. Simple trigonometry shows that for light incident at an angle θ onto parallel atomic planes separated by a distance d, the first peak of diffraction takes place for wavelengths λ given by Bragg's law:

$$\lambda = 2d \sin\theta. \tag{1}$$

It is important to note that (1) relates a physical dimension of the illuminated material with the wavelength of the light.

For very short X-ray waves with wavelengths of the order of $10^{-10}m$ Bragg's effect takes place at the atomic level. Diffraction photographs of crystals produced very ordered patterns proving the existence of a very particular arrangement of the atoms in the material. In crystallography one has to deal with an inverse problem. The structure of a crystal, or at least its symmetries, is to be determined by looking at the crystal spectrum, i.e., the patterns in their Fourier transforms.

In biological tissues, structural color production takes place at a much larger spatial scale than the inter atomic distance in crystals. Nevertheless, the situation is similar to Bragg's law. As expressed by Benedek in [1], it is a general principle that

> "*. . .light is scattered only by those fluctuations in the index of refraction whose wavelengths are larger than one-half of the wavelength of the light in the medium.*"

The structure in the material originating those fluctuations can clearly be observed in electronic microscope images of the tissues, and their Fourier spectrum can be computed and related to the spectral measurements made with a spectrophotometer. Essentially, the predominant spatial periodicity of the tissues, as quantified by the Fourier transform, gives a prediction of the wavelengths of light scattered the most. The direct problem of computing the Fourier transform is simpler than the inverse problem of crystallography and can be carried out numerically using the fast Fourier transform (FFT). (But this truth has interesting biological implications, i.e., there are multiple biological nanostructures that can make the same color!)

1.3 More About Color

In describing colors and forms of coloration it is convenient to recall the difference between the production of color by addition or subtraction, which sometimes produces some confusions. Coloration by addition is the result of the combination of light of different wavelengths. For example, the superposition of red and blue lights over a white screen produces the so-called color magenta. If one adds light of its complementary color, green, one obtains white light. On the other hand, the coloration produced in the presence of pigments is due to color subtraction. The color attributed to a pigment, the one observed, is the one complementary to the one absorbed. For example, if we mix a green pigment (one that absorbs blue and red) with a magenta one (one that absorbs green) the result is black.

It is important also to recall that the visible spectrum of humans ranges approximately between 400 nm and 700 nm. Our optical systems posses three color

receptors most sensible to different sets of wavelengths around the red, green, and blue colors. It is the combined excitation of these receptors together with the amount of luminosity and ambient light conditions that determines the final interpretation of colors that our brain makes of certain electromagnetic waves. The colors observed in birds due to coherent scattering result from the constructive superposition of the wavelengths scattered the most. In the study of the resulting hues observed and measured by spectrophotometry, the rules of coloration by addition take place. However, unlike our ears, which let our brain distinguish between each individual note played as part of a cord, our visual system only interprets the final result of the superposition of light of different wavelengths. That is, the same perceived color can be created by addition in different ways.

It is interesting to note that birds have a broader visible spectrum with a fourth receptor and are able to see into the UV (320–400 nm) part of the electromagnetic spectrum [11]. It is perhaps impossible for us to image how do the colors seen by birds actually look like to them because of this ability to see UV ones, but we can still study the full spectral content of the signals. This detailed spectrum, undetected by our eyes but measurable by a spectrophotometer and predicted by our Fourier analysis, is what helps us explain the physical mechanisms taking place in the production of the color.

In the rest of this expository article we chose to describe some of the physical and mathematical models employed in the description of structural colors in the skin of some birds. The same models apply to feathers and other living tissues. We refer to the already cited literature for more technical details. This note also overlap in part with a more elementary exposition translated into Spanish presented in [32].

2 A Physical Model for Coherent Scattering

To explain how Fourier methods can be used to predict the color produced, we based our analysis on some of the work in [1, 33, 34] and the references therein. A mathematical and physical explanation of the transparency of the human cornea (a biological tissues similar in structure to the wattles of some birds), as well as the reasons of its turbidity due to swollen pathological abnormalities, was given by Benedek in [1]. The cornea is made of long and thin parallel collagen fibers immersed in a ground substance of mucopolysaccharide. A cross section of a bundle of such fibers looks very much like the cross section of the tissues of some birds, though at a smaller scale. According to Benedek, Maurice [14] was one of the first researchers to realize that, to explain the transparency of the cornea, it was important to understand the relationship among the phases of waves scattered by each of the fibers in the tissue. Maurice first speculated that the fibers should be equal in diameter and have their longitudinal axis centered on the points in a perfect lattice. Maurice thought the absence of the perfect crystalline periodicity in electron micrographs of the cornea was an experimental artifact, but soon it was realized that the corneal collagen fibers were not arranged in a perfect crystal lattice which

required a new theoretical explanation. A series of experimental, numerical, and theoretical works culminated then with Benedek's explanation that a perfect lattice arrangement is not necessary. Again, fluctuations in the index of refraction whose wavelength are equal to or larger than one-half the wavelength in the medium of an incident light are responsible for most of the coherently scattered light. In the case of the cornea these fluctuations from the fibers to the ground substance in which they are immersed are of very small physical dimensions and produce most of the scattered energy at very small wavelengths [33]. Wavelengths in the visible part of the spectrum are then almost completely transmitted, giving the transparency of the cornea.

The fibers in the tissue can be modeled as very long and thin cylinders or rather needles. Benedek described the propagation of a scattered electromagnetic field in the plane perpendicular to these fibers. Because of the particular geometric arrangement, further physical considerations imply that most of the scattered field by each fiber propagates only in this plane, and a two-dimensional analysis is a reasonable approximation to the physical situation. A brief and simplified description to illustrate the arguments in [1] is as follows. To model the situation, imagine then a distribution of point masses M_j at positions x_j in the plane and an incident light wave

$$E(x,t) = E_0 \mathrm{e}^{\mathrm{i}(k_0 x - \omega t)}, \tag{2}$$

where the two-dimensional wave vector k_0 has length

$$|k_0| = 2\pi\eta/\lambda, \tag{3}$$

η is the mean index of refraction, λ is the wavelength of the incident beam, and ω is the angular time frequency of the incident light. The incident electric field induces oscillating dipoles in the medium which in turn irradiate new electric fields in every direction, and also part of the field is transmitted. The scattered field at a particular position in the plane is determined by the superposition of all the individual scattered fields. The rays emanating from different fibers travel different distances to a given fixed point. Moreover, the oscillations induced by the incident field at the different positions R_j take place at different times producing also a retardation in time. Appropriately using this time delay and path addition, the field scattered by M_j at a position R in the direction given by the vector k_R, with $|k_R| = |k_0|$ and forming an angle θ with the incident wave, is computed in [1] to be

$$E_j = E_0 \mathrm{e}^{\mathrm{i}(k_0 R - \omega t)} \mathrm{e}^{-\mathrm{i}k x_j}. \tag{4}$$

Here $k = k_0 - k_R$ is called the scattering vector and

$$|k| = 2|k_0| \sin(\theta/2) = \frac{4\pi\eta}{\lambda} \sin(\theta/2). \tag{5}$$

Or, in terms of wavelengths, we have that

$$\lambda = 2\eta\lambda_k \sin(\theta/2), \tag{6}$$

where

$$\lambda_k = \frac{2\pi\eta}{|k|}. \tag{7}$$

The total scattered field is then given by

$$E_T = E_0 \mathrm{e}^{\mathrm{i}(k_0 R - \omega t)} \sum_j \mathrm{e}^{-\mathrm{i}k x_j}. \tag{8}$$

Note that, formally using delta distributions and the Fourier transform, the last factor in (8) is the interference function which can be seen as a Fourier transform

$$I(k) = \sum_j \widehat{\delta}_{x_j}(k) = \widehat{(\Sigma_j \delta_{x_j})}(k) = \widehat{f}(k), \tag{9}$$

and where

$$f = \sum_j \delta_{x_j} \tag{10}$$

can be viewed as a density distribution of mass.

The intensity of the scattered light is proportional to the square of the scattered electric field. Thus, as argued by Benedek, the intensity will be large for those spacial frequencies k so that

$$|I(k)|^2 = |\widehat{f}(k)|^2 \tag{11}$$

is large. For example, when we measure backward scattering (that is the one back to a distant observer) which correspond $\theta = \pi$, the scattering will be very intense if f has a large Fourier component with wavelength

$$\lambda_k = \lambda/(2\eta), \tag{12}$$

i.e., *half the wavelength of the wavelength in the medium of the incident light.* This is a restatement of Bragg's law in this context, which permits again to relate the wavelength of the constructively reinforced scattered light with a physical dimension in the material.

Like with crystals or quasicrystals, if the density function f is very *ordered*, then the Fourier transform $\hat{f}$ will show clear *peaks* at certain frequencies. Loosely speaking (see [29]), a quasicrystal can be defined to be a countable set Λ such that there exits another (dual) set $\widehat{\Lambda}$, with the property that

$$\widehat{(\Sigma_{x_j \in \Lambda} \delta_{x_j})} = \sum\nolimits_{y_j \in \widehat{\Lambda}} \delta_{y_j} + \text{“small continuous spectrum.”} \tag{13}$$

When computed numerically the size of the Fourier transform of a quasicrystal reveals very high values at the (approximate) positions in the set $\widehat{\Lambda}$ with the continuously distributed spectrum as a background noise.

The physical description we gave above is only an approximation of the real situation. In the case of many tissue we no longer have very thin needles or even fibers. The density f in Benedek's theory is then replaced by the fluctuations in density from an average value. We refer again the reader to [1]. The density function f can be seen in the electronic microscope observations of the tissues. The predominant components in the Fourier transform of the density function are what we still claim determine to some extend and via (12) the hue and the distribution of energy observed in the spectrophotometer.

In tissues that lack a perfect crystal structure, the observed peaks will not necessarily be on a lattice, but they will still occur around a certain characteristic frequency within the visible spectrum. To determine theoretically the exact position of such peaks would require a precise knowledge of the dimensions and arrangements of the fibers. Because of the diversity of tissues and variations in specimens making assumptions about the exact diameter and position of fibers is too rigid to model many real-life situations, and we perform instead a numerical calculation of the Fourier transform. It is not possible to characterize all functions which will produce a noticeable peak in their spectra within a certain bandwidth. We are only interested in a particular scale that affects the distribution of energy of the scattered light in the visible part of the spectrum. What we want to corroborate is that the numerous tissues examined do posses the necessary order to produce such peaks.

3 Fourier Analysis of Nano-Structured Tissues and Color Prediction

We illustrate this application of Fourier analysis with some results already in the literature. Our first study on bird's skin was from the brilliantly colored patches around and above the eyes of a small group of perching birds from Madagascar—the asities (Eurylaimidae, Aves)—shown in Fig. 1 which we reproduce from [23]. As described in [23] the asities are a group of suboscine perching fruit and nectar-feeding birds endemic to the tropical forests of Madagascar. Adult males of the asities have brilliantly colored, sexually dimorphic facial skin during the breeding season. The colorful patches of facial skin play an important role in inter-sexual communication and mate choice of these birds.

The caruncles in the dermis of these tissues (Fig. 1b) are composed of numerous bundles of macrofibrils arrays of long parallel collagen fibers of similar diameters and separated by a mucopolysaccharide matrix. At large scales, the macrofibrils have little apparent order and run through the tissue in different directions. However, cross sections at 10k–50k magnification of any macrofibril reveal the circular shape of the cross sections of the parallel collagen fibers and their uniform distribution.

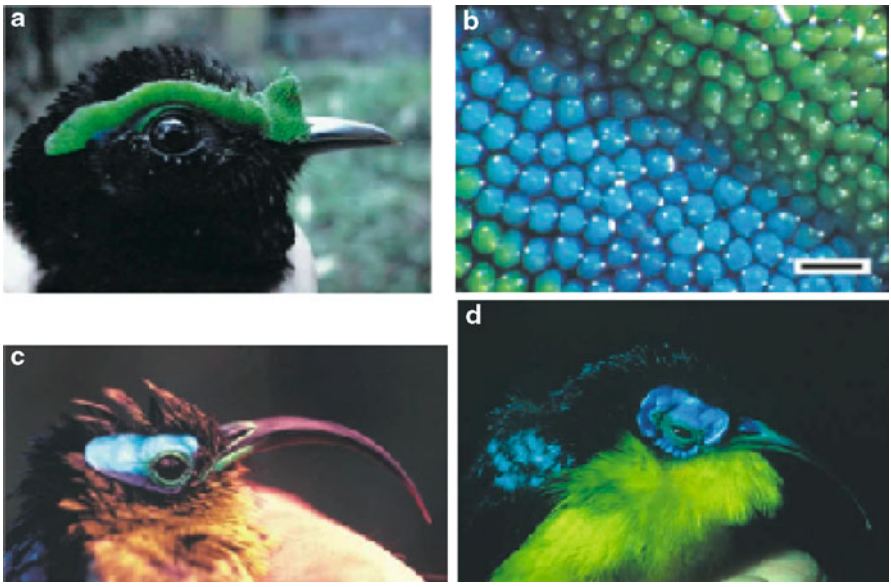

Fig. 1 *Blue and green* facial caruncles of asities. (**a**) *Phileipitta castanea*. (**b**) Close-up of the supraorbital caruncles of *Phileipitta castanea*. Scale bar approximately 500 μm. (**c**) *Neodrepanis coruscans*. (**d**) *Neodrepanis hypoxantha*

Figure 2 (also reproduced from [23]) shows a cross section of the collagen fibers of a typical tissue and the corresponding (modulus square of the) FFT of the image. The fibers show small variations in their diameters and center- to- center distances. The fibers in this image are not arranged in a crystal-like array, and the Fourier transforms shows certain concentric ring structures and have a radial-like symmetry (although it obviously cannot be perfectly radially symmetric). The intensity of the rings decreases as we move away from the origin on the Fourier transform domain. Intuitively the images can be thought as being made up of certain predominant periodicities of a particular length in every direction. The location of peaks in the side of the Fourier transform indicates that the fluctuations in the density function are rather homogeneous and similar in all directions in the tissues at least at a particular small scale. This is clearly observed in the images of the tissues. We call this arrangements in the tissue a *quasi-order*. The distance between nearest neighboring fibers does not change much from place to place, though there is very little correlation among fibers that are further away.

A big diversity of tissues and their corresponding FFTs can be seen in Figs. 3 and 4 below. They are from a larger study of many other birds that we carried out in [20]. Interestingly, some tissues analyzed do present an almost perfectly periodic structure which is clearly present too in the FFT of the images of the tissues. Note, for example, the image of tissue from *Philepitta castanea* (bird photo in Fig. 1a),

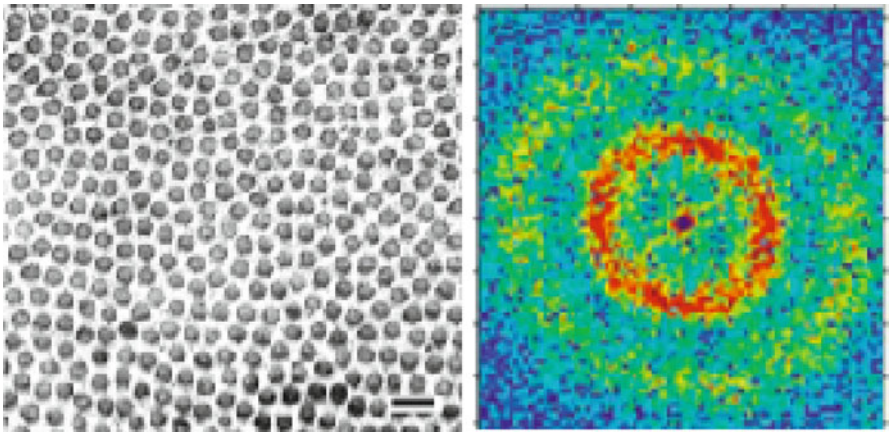

Fig. 2 Typical transmission electron micrograph of a cross section of an array of collagen fibers from the caruncle tissues. Scale bar approximately 200 nm. The colors map from *blue* to *red* indicate the magnitude of the squared Fourier components. *Blur* indicates small values and *red* high ones

which is given in Fig. 3i, and its FFT given in Fig. 4f. (For photos of the other birds mentioned in the figures please see [20].)

What is observed in the FFT images can be explained as follows: The radially decay of intensity is determined, in part, by the fact that the fibers are not needles whose cross sections are determined by delta masses at certain points but rather have approximately circular cross sections of a certain radius R. Though the physical problem is different, the mathematical problem of computing the Fourier transform of such collection of circles (or rather the characteristic function of the cross section) is equivalent to determine the Franunhofer diffraction patterns produced by a number of circular apertures of similar size.

Let B_0 be a circle of radius R centered at the origin in two-dimensional Euclideian space and let χ_{B_0} be its characteristic function. Let B_j be the circle with same radius but with center translated to the point x_j and let χ_{B_j} be the corresponding characteristic function. The Fourier transform of a the images of the tissue is then the Fourier transform of the characteristic function of a collection of circles $\{B_j\}_{j=0}^N$. Using the properties of the Fourier transform this is easily computed to be

$$\widehat{\Sigma_{j=0}^N \chi_{B_j}}(y) = \hat{\chi}_{B_0}(y)\, \widehat{\Sigma_{j=0}^N \delta_{x_j}}. \tag{14}$$

Therefore, the Fourier transform is determined by the product of two factors. One is determined by the shape of the aperture, while the other is determined only by the position of them. For a circle, the first factor is a radially decaying or damped wave. The sum of deltas in (14) is part of what is sometimes called a Dirac comb. For appropriate distribution of the deltas, the modulus square of the Fourier transform of them presents very high peaks (almost new deltas) at a particular position.

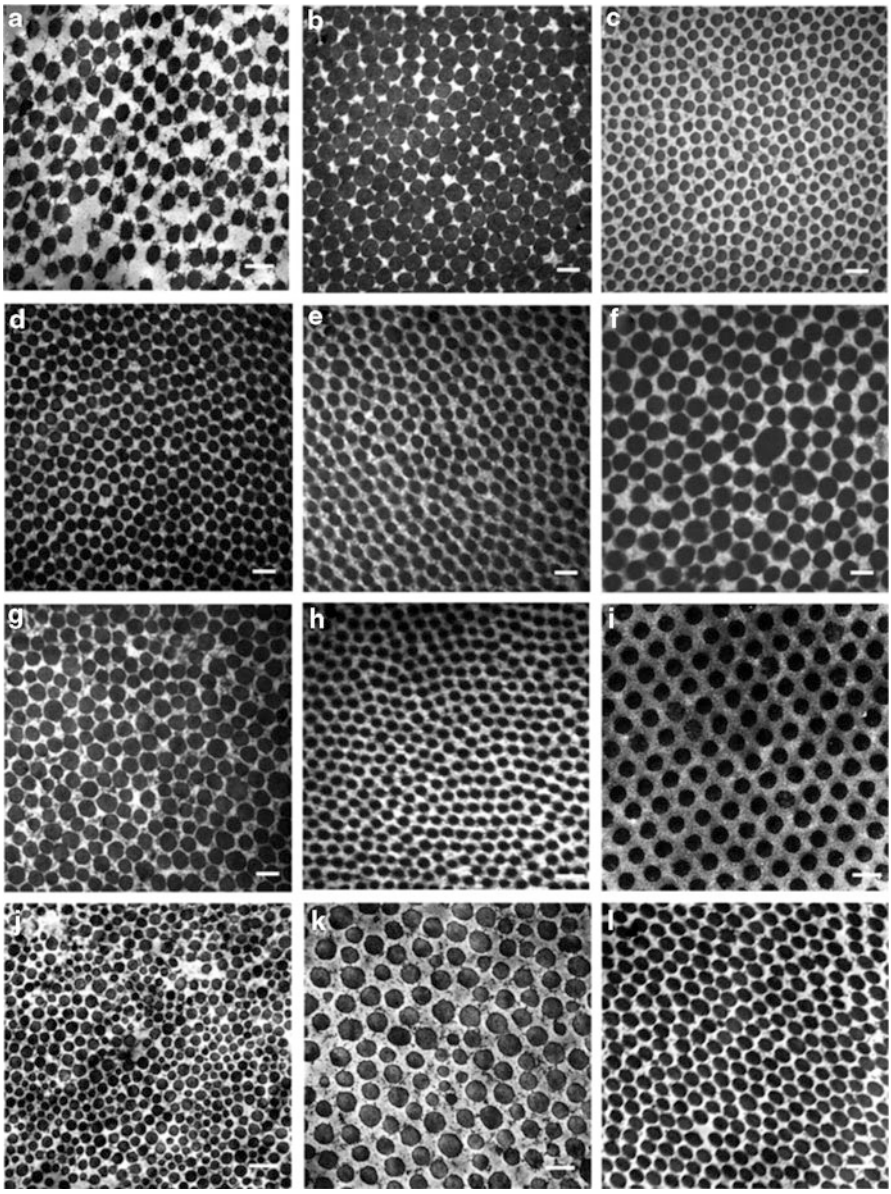

Fig. 3 Transmission electron micrographs of nano-structured arrays of dermal collagen from several species of birds of different colors. (**a**) *Oxyura jamaicensis*, *light blue*; (**b**) *Numida meleagris*, *dark blue*; (**c**) *Tragopan satyra*, *dark blue*; (**d**) *Tragopan caboti*, *dark blue*; (**e**) *Tragopan caboti*, *light blue*; (**f**) *Tragopan caboti*, *orange*; (**g**) *Syrigma sibilatrix*, *blue*; (**h**) *Ramphastos toco*, *dark blue*; (**i**) *Philepitta castanea*, *light blue*; (**j**) *Gymnopithys leucapsis*, *light blue*; (**k**) *Procnias nudicollis*, *green*; and (**l**) *Terpsiphone mutata*, *dark blue*. All scale bars represent 200 nm

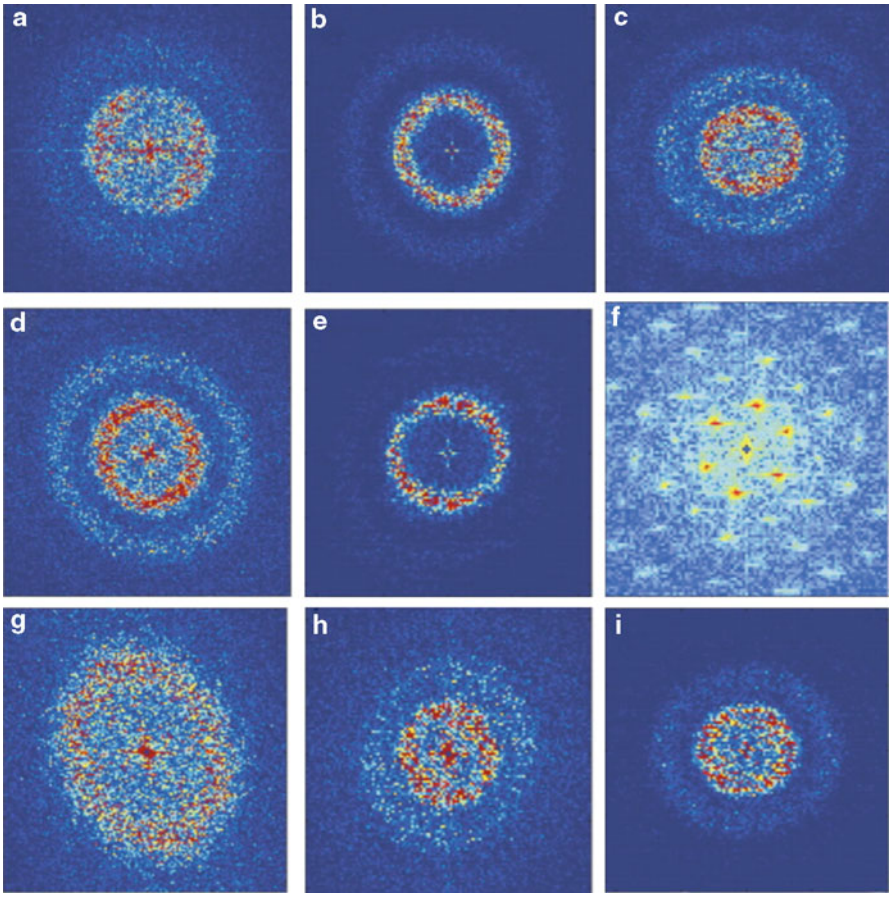

Fig. 4 Two-dimensional FFT spectra of transmission electron micrographs of nano-structured collagen arrays from the tissues of different birds. (**a**) *Dromaius novaehollandiae*, *blue*; (**b**) *Tragopan satyra*, *dark blue*; (**c**) *Pilherodius pileatus*, *light blue*; (**d**) *Coua reynaudii*, *dark blue*; (**e**) *Ramphastos toco*, *dark blue*; (**f**) *Philepitta castanea*, *light blue*; (**g**) *Gymnopithys leucapsis*, *light blue*; (**h**) *Procnias nudicollis*, *green*; and (**i**) *Dyaphorophyia concreta*, *yellow green*. The colors from *blue* to *red* indicate the magnitude of the squared Fourier components

For example, if we consider an infinite dimensional lattice of points in two dimensions generated by linear combinations with integer coefficients of two linearly independent vectors $\boldsymbol{v}_1$ and $\boldsymbol{v}_2$, then the Fourier transform of the sum of the deltas at the points of the lattice is a sum of deltas at a dual lattice. This fact is just a restatement of Poisson summation formula. See, for example, [29] or [3]. The dual lattice is generated by the vectors $\boldsymbol{u}_1$ and $\boldsymbol{u}_2$ satisfying $\boldsymbol{u}_k \cdot \boldsymbol{v}_j = \delta_{kj}$, where now δ_{kj} is the Kronecker delta, $\delta_{jk} = 0$ if $j \neq k$ and $\delta_{jj} = 1$. If $A = (\boldsymbol{v}_1, \boldsymbol{v}_2)$

is the matrix of the linear transformation that maps the standard square lattice onto the lattice generated by $\boldsymbol{v}_1$ and $\boldsymbol{v}_2$ then $(A^{-1})^* = (\boldsymbol{u}_1, \boldsymbol{u}_2)$, where * denotes the transposed of a matrix. See again [29] and [3] for details.

If we consider instead a finite portion of an infinite lattice and compute its Fourier transform, one observes distinct peaks at the points of the dual lattice rather than deltas, but an *echo*, as called in [29], or a *finite size effect* is observed as a variation in intensity. The location of the peaks is not affected much by this echo, but the height and width of the peaks are. In particular, the height is determined by the number of points in the finite region of the lattice analyzed and, hence, in our case, the physical length of the image of the tissue. In a perfect lattice as the number of points increases to infinity the Fourier transform converges locally around the peaks to delta distributions. However, when analyzing biological tissues, the finite effect should not be completely disregarded because the tissues do have specific finite dimension.

In the quasi-ordered tissue a precise mathematical description is harder to state. Except for the local order extended to the next neighboring fibers, the tissues have no order that can be analytically quantified in an obvious way. To quantify such order or (lack of it) we compute the Fourier transform numerically. Intuitively, the lack of order at larger distances makes the predominant frequencies to be mostly associated to the nearest-neighbored order, and the quasi-homogeneity of the tissues (the tissues look the same in any orientation) makes the peaks in the side of the Fourier transforms to be uniformly distributed in a ring at particular frequencies. The peak at the origin of the Fourier transform corresponds to the transmitted energy of the incident field that is not scattered. The first peak outside the origin occurs at a frequency determined in part by the average distance from the center of a fiber to the center of the nearest one and the size of the fibers and the overall arrangement, and represents the main physical periodicity in the tissue.

Using Benedek's theory we can try to use the Fourier transforms of the images of the tissues to give some prediction of the dimensions of the spatial variation in refractive index and hence the predominant wavelengths to be constructively scattered. The refractive indices of the collagen and the mucopolysaccharide are known (approximately 1.55 and 1.35, respectively). With these indexes of refraction and the density function as observed in the electronic microscope image, the average refractive index used can be estimated numerically from the micrographs (by looking at regions of black and white). Using the peaks observed in the FFT of the image of the tissue, one can predict the wavelength of the predominant color observed using the formula (12). For backward scattering, wavelengths of about twice the spatial periodicity measured by the FFT will be scattered the most.

It is not only the peaks in the Fourier transform what matters in the model but also the general distribution of energy. In order to exploit further information encoded in the Fourier transform of the images, we want to make some kind of comparison of the distribution of energy of our predictions with the actual spectrophotometer measurements. As mentioned in the introduction colors can be made up in different ways, but the whole spectral distribution helps falsify the Rayleigh hypothesis.

For the comparison, we first need to have a one-dimensional distribution of energy as the one given by the spectrophotometer. One can think of several ways to do this. One is, for example, to select an arbitrary radial direction. A similar approach to this was carried out by Vaezy et al. [33, 34]. However, the radial symmetry of the Fourier transform of the quasi-ordered tissues suggests that we consider instead a different analysis. For the comparison we want to further accentuate the radial symmetry, and hence we replace $|\widehat{f}|^2$ by its average on small concentric rings. Though artificially imposed, this radial (or azimuthal) average certainly reflects the ringlike structure observed in the images of the Fourier transform of the quasi-ordered tissue. To obtain a one-dimensional distribution of energy we use the radial average distribution to compute the total energy in each frequency band. We normalize the total energy or Fourier power (the L^2 norm of the Fourier transform) to be one over the visible part of the spectrum. Finally we plotted the amount of energy on a certain bandwidth as a percentage of the total energy and compare it with the spectrophotometer measurements (reflectance spectrum).

Figure 5 reproduced from [20] shows the comparisons between the actual reflectance spectrum measured with a spectrophotometer and our predictions using the radial average FFT. These were done for several tissues whose images appear in Fig. 3 and whose FFT are given in Fig. 4. See also [20, 23] for further technical details.

We observe that the resulting general profile of distribution of relative energy is similar to the one obtained by spectrophotometry. The quantitative discrepancy in the actual numerical computation is not surprising given the many elements involved in the collection and preparation of the specimens that could slightly modify their structure and the numerous approximations and analytical simplifications we have made, both physically and mathematically. The qualitative similitudes in the observed and predicted spectrum (both in terms of locations of peaks and general shape) are, on the other hand, quite noticeable and are a reasonable experimental corroboration of the validity of the physical model used. It is evident that only certain wavelengths are coherently scattered, and their range of values (and hence the colors observed) are determined by the physical periodicities in the tissues.

4 Lack of Iridescence and Other Works that Followed

Our experiments also clearly put in evidence that the color is not produced by Rayleigh scattering (which would produce for spectrum a ramp toward the UV). Our analysis based on the existing theory by Benedek was the first to provide an alternative explanation for the phenomena. Further, the general radial symmetry of the Fourier transform of the quasi-ordered tissues explains why these tissues are not highly iridescent: the spatial frequency of variation in refractive index remains similar in all directions within the quasi-ordered tissues and thus produces a uniform hue for backward scattering of light independent of the angle of incidence. (See

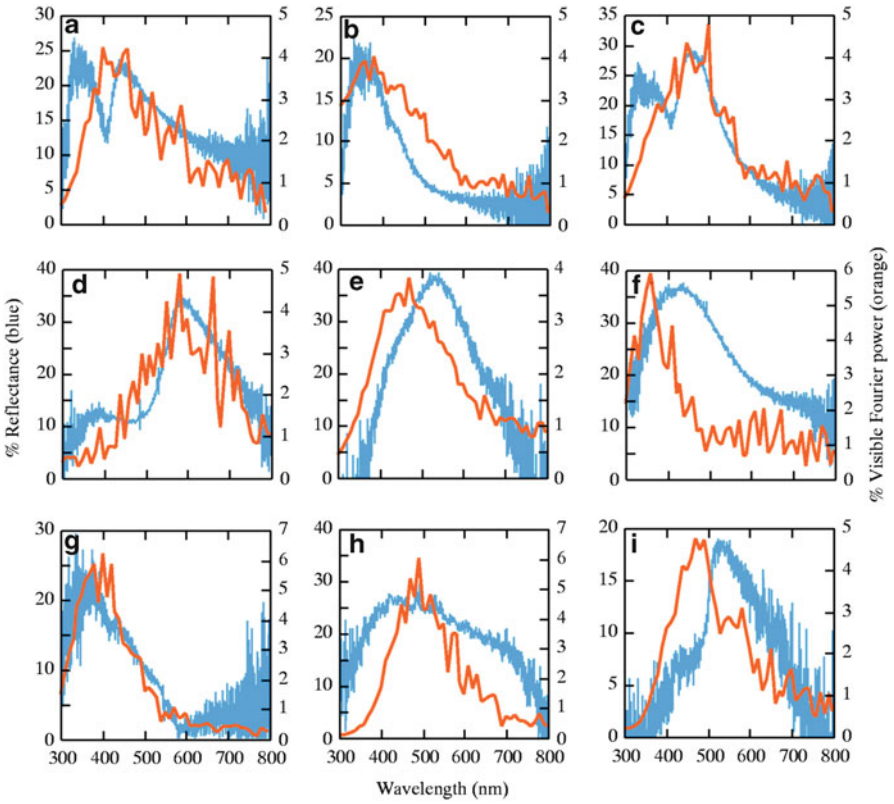

Fig. 5 Comparisons of reflectance spectra (*blue*) measured with a spectrophotometer and the Fourier-predicted spectra (*orange*) for samples of tissues from different birds. (**a**) *Lophophorus impejanus, dark blue*; (**b**) *Tragopan temminckii*, *dark blue*; (**c**) *Tragopan temminckii*, *light blue*; (**d**) *Tragopan caboti, orange*; (**e**) *Syrigma sibilatrix*, *light blue*; (**f**) *Coua caerulea*, *dark blue*; (**g**) *Ramphastos toco*, *dark blue*; (**h**) *Selenidera culik*, *green*; and (**i**) *Dyaphorophyia concreta*, *yellow-green*. The reflectance spectra are reported as a percentage reflectance (*blue*, *left axis*), and the predicted spectra are reported as a percentage of Fourier power (*orange*, *right axis*)

Noh et al. [17] for a rigorous demonstration of this fact.) Interestingly, it was this feature of coherent scattering from quasi-ordered materials that originally led to the confusion with Rayleigh scattering. Researchers in the field traditionally conceived of only two alternative sorts of order: complete crystalline periodicity or complete random distribution of particles. Within this framework, iridescent structural colors were associated with the interference from crystalline materials, and non-iridescent colors were associated with Rayleigh scattering from random distributions. The possibility of order only at the local scale and its optical consequences were not considered.

In very ordered materials to perform the radial average is, perhaps, not fully justified, but the relative intensity of the peaks is so large that we still get a good

match with the measured reflectance spectrum, see again [23]. In fact, the color of the extremely ordered tissues is more brilliant and *pure tone* than those in some of the quasi-ordered ones. A natural question to ask at this point is why then the very ordered tissues are non-iridescent. The answer lays again in the more complicated structure of the tissue at other larger scales and which is hard to incorporate in our first analysis. As mentioned before, the tissue is made of several layers of fibers in different directions and, as explained in [1], the total scattered field is some average field made from the contributions of all the layers. The lack of organization of the different layers has a similar effect to what is already observed in the quasi-ordered tissue. Though within an array of parallel fibers running in a particular direction we have an almost perfect hexagonal lattice, the same lattice in another set of parallel fibers may appear rotated by an arbitrary angle. The total intensity then will have a substantially uniform peak in its frequency content in a dense distribution of angles around the origin. An average effect is still what we observe or measure with the spectrophotometer. In other words, if all the cross section of parallel arrays of fibers would have the same orientation for the observed hexagonal lattice, the tissues would be iridescent. But, as we just explained, this is not the case. For comparison, we mention again hummingbirds, whose structurally color tissues have an almost perfect parallel laminar morphology resembling thin parallel films, and hence they do produce iridescent coloration according to Bragg's law.

Blue, green, and violets produced by coherent scattering have been documented by now by our methods. One could speculate that warmer colors are harder to produce by constructive interference since they would require a spatial order at a larger scale, which is perhaps too difficult to achieve in a biological tissue that needs to keeps such order as it grows. Otherwise, it may be that rarity of blue or green pigments prevents animals from making pigmentary blues or greens, but that the availability of long wavelength pigments favors those outcomes.

We have also analyzed feather barbs which look black to human eyes but possess vivid UV peaks (approximately 350 nm) that are not visible to humans but are easily perceived by birds. See [25]. In feathers the periodicities in the tissues take place in three dimensions and are provided by a distribution of air bubbles inside the tissues.

The methods have also been applied with similar results to the study of coloration in primates [21], butterflies [27], and dragonflies [26]. In addition, the Fourier method has been applied by Shawkey et al. [30] to a three-dimensional bird feather data set that was acquired by electron tomography. The empirical result provided some advance over 2D Fourier analysis of electron micrographs when dealing with 3D arrangements, but it still had inaccuracies due to systemic distortions in the 3D tomographic reconstruction.

The works mentioned above were some of the first approaches to the understanding of so many diverse structurally colored biological tissues. Since then many other works have appeared in the literature. The results using the relative simple model of Benedek have been by now corroborated with other more comprehensive techniques and experimentation. In particular, Prum and a multidisciplinary team at Yale University have recently employed in their studies small-angle X-ray scattering (SAXS) carried out at the Argonne National Labs. These studies hope to improve

upon the empirical limitations of Fourier transforms of electron micrographs by direct measurement of the Fourier transform of electron density variations in these nanostructures. See the works [4,5,12] for technical details and further explanations.

In our original analysis unidirectional light was assumed, and we were concerned only with backward scattering. A more delicate analysis and experimentation using omni-directional lighting in the quasi-ordered structures of birds feathers was recently carried out in [17] using SAXS. It was shown in [17] that in fact, under directional light, the scattering peak occurs in the backward direction. Moreover the authors in [17] also showed that under omni-directional lighting the colors observed remain unchanged with the angle of observation. See the cited reference for further information.

Likewise, our original analyses only concerned single scattering, i.e., interactions of photons that were each scattered only a single time by the scattering objects. But it became apparent that some inaccuracies in experimental comparisons of Fourier predicted and measured reflectance spectra were the result of multiple scattering: i.e., interactions among photons scattered two or more times by the nanostructures. This led to new physical theory and tests on double scattering by quasi-ordered nanostructures [16,18]. These works show that multiple scattering by quasi-ordered nanostructures produces new optical phenomena (e.g., double-peaked reflectance spectra) that were not anticipated in traditional optics. Although they require a new experimental method, the new X-ray scattering studies demonstrate the fundamental relevance and accuracy of the Fourier transform to the analysis of this optical phenomenon in nature.

The study of nature made structured tissues also relates to the study of photonic materials. A lot of activity in this area has taken place as groups of researchers try to fabricate photonic crystals and understand their properties. See [8] for references. In addition the tissues we studied have resemblances and similar physical properties to *hyperuniform* systems as studied, for example, by Torquato and Sillinger in [31]. These systems are theoretical arrangements of distribution of points that produce complete band gaps at low frequencies. The understanding of the fluctuation of density in materials and their scattering and transmitting properties will certainly continue to be an intense area of research in the immediate future. It is interesting to see how such materials are already present in biological tissues and are used in nature for a variety of purposes.

5 Summary

We wanted to illustrate here how Fourier analysis and numerical experiment with numerous tissues sustain the claim that quasi-ordered systems can produce non-iridescent structural colors by coherent scattering. Such color production occurs when only some wavelengths of visible light are selectively reinforced. The Fourier transform becomes an ideal analytical tool because it is a mathematical analog of the actual physical process of light interacting with the optically heterogeneous tissues.

The application presented renewed our appreciation of the ability of the Fourier transform to codify order or the lack of it, which makes Fourier analysis a very valuable tool for studies in material sciences.

Lastly, we find the use of Fourier analysis in biological questions addressing physical phenomena that affect communication and behavior in animals rather thought-provoking. We marvel at this beautiful manifestation of Fourier analysis in nature and the role it may play in sexual selection in many bird species. In fact, the animals' sexual preference for a specific color is not really based on the physical reason for the coloration, which is the collagen fiber order at invisible nano-scales. Instead, preference is based on the observable features of the reflectance spectrum resulting from such order. We can say that, essentially, preference is based on the Fourier transform of the invisible structures!

As it is well-known, Fourier introduced his groundbreaking analysis of the heat equation (by now called Fourier analysis) in his famous *Analytic theory of heat* [9]. We have mentioned in other occasions (e.g., [32]) a favorite quote from his work, which we want to repeat here one more time:

> "*...if the order which is established in this phenomena could be grasped by our senses, it would produce in as an impression comparable to the sensation of musical sound.*"

With this quote in mind, we would like to conclude by pointing out that the order in the nanostructures of the biological tissues studied can indeed be perceived by our senses as vivid colors, and these colors can certainly be as aesthetically pleasing to the observer as the sensation of musical sound referred to in Fourier's words.

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