Optical probing of long-range spatial correlation and symmetry in complex biophotonic architectures on transparent insect wings

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Abstract
We experimentally probe the structural organization of complex bio-photonic architecture on transparent insect wings by a simple, non-invasive, real-time optical technique. A stable and reproducible far-field diffraction pattern in transmission was observed using collimated cw and broadband fs laser pulses. A quantitative analysis of the observed diffraction pattern unveiled long-range quasi-periodic order in the arrangement of the microstructures over mm scale.
These observations agree well with the Fourier analysis of SEM images of the wing taken at various length scales. We propose a simple quantitative model based on optical diffraction by an array of non overlapping microstructures with minimal disorder which supports our experimental observations. We observed a rotation of the original diffraction profile by scanning the laser beam across the wing sample which gives direct signature of organizational symmetry in microstructure arrangements at various length scales. In addition, we report the first optical detection of reorganization in the photonic architecture on the Drosophila wings by various genetic mutations. These results have potential for the design and development of diffractive optical components for applied photonics and may open up new opportunities in biomimetic device research.

Keywords: natural photonic structures, diffraction, Fourier optics, nanophotonics and photonic crystals

(Some figures may appear in colour only in the online journal)

1. Introduction
Nature has developed a remarkable variety of photonic structures in various insect wings [1–3]. The cooperation of structural heterogeneities (regularity and irregularity) [4, 5] in these natural bio-photonic architectures at optical wavelength scale interact with light in a specific way to produce various optical effects such as reflection [6], interference [7], diffraction [8], fluorescence [9], iridescence [10], and polarization sensitivity [11]. Compared to the equivalent man-made optical devices, the biophotonic structures often possess greater complexity and could outperform their functions in some cases [2, 12]. Due to the presence of multiple length scales and the diversity in their design, the optical behavior of such arrangements is still not fully understood.
While the optical effects in the non-transparent wings of butterflies and beetles have been well studied [3, 5, 11, 13], the
transparent wings of many insects (Drosophila, bees, dragonflies) have attracted much less attention. Previous experiments on thin transparent wings observed various interference colors under white light illumination and quantified their transmission and reflection spectra [14, 15]. Recently, the transparent wings of fireflies have been exploited in designing and optimizing optical components such as anti-reflection elements in laser diodes [16]. High resolution techniques like scanning electron microscopy (SEM) and atomic force microscopy have resolved the structural complexity of transparent wings (of dragonflies) at micro and nano-scale [17]. These studies revealed that the wing surface is decorated with a large number of micro-structures [3, 13, 15, 23]. These microstructures were known to provide anti-wetting, self-cleaning and aerodynamic properties to the wing surface [28–30]. Recently, modulation of friction and adhesion on these microstructures was observed [31]. However, the long-range organizing principles and symmetry of the complex photonic architecture in transparent wings remain unexplored. A knowledge of their organization would be fundamental to understand how these systems have been naturally optimized to coherently manipulate light for various functions [18, 19].

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The aim of this letter is to address the following questions: is there any long range (mm scale) order and organizational symmetry in the array of microstructures on the transparent insect wing? Can we exploit the complex diffraction pattern to quantitatively unveil new features in the arrangement of microstructure array? Answering these questions is crucial to understanding the design principles and multi-functional role of transparent wings [17, 19, 31]. The letter is organized as follows. In section 2, we describe our experimental set-up. In section 3, we report experimental observations of the diffraction pattern using various lasers. We give a theoretical interpretation of these results using SEM analysis of the samples. In sections 4 and 5 we demonstrate applications of the optical techniques by measuring the correlated diffraction pattern at various scales in the wing and by quantifying the role of genetic mutations on the photonic architecture of the transparent wings of the Drosophila.

2. Experimental set-up

A schematic diagram of our experimental set-up and its actual picture is shown in figure 1. In our set-up a collimated laser beam passes through a wing sample that is mounted on a xyz micrometer translation stage. The transmitted laser intensity was captured through the wings by a digital camera and analyzed.
We used both monochromatic cw lasers at two visible wavelengths in red and green ($\lambda = 532$ nm and 632 nm) and femtosecond pulses centered in near IR range 800 nm. These wavelengths are chosen to match the transparency window of our wing sample. The typical intensity transmission coefficient of our insect wing is around 60% at these wavelengths. The $1/e^2$ full-waist of the collimated laser beams was around 1 mm which is much smaller than the typical wing size >1 cm. The input beam profile of these lasers is shown in figure 2. The far-field diffraction pattern was captured on a white screen fixed at $D = 20.5$ cm from the wing sample. We have observed that the diffraction pattern is fully developed after few cm from the wing and it simply diverges thereafter due to geometrical effect. It is worth mentioning that with this simple set-up, no preparation of the wing sample is required. In fact, it can be used for *in vivo* non-destructive imaging of the wing with the insect alive. The laser powers were very low and no sign of optical damage was seen on the wing surface. To validate the sensitivity of our optical technique we also performed SEM images of the wing surface (figure 3). The high resolution SEM images showed that the wing surface is decorated with a large number of non overlapping microstructures. The flat background of the wing exhibits nano-grain-like features which are typically smaller than the wavelength of the light. These microstructures were elongated having typical length and width around 5–8 $\mu$m and 1–2 $\mu$m, respectively. Note that their dimensions are comparable to the

3. Results and discussion

3.1. Observation and analysis of complex diffraction pattern

Remarkably, a collimated laser beam ($1/e^2$ full waist around 1 mm) formed a stable and characteristic diffraction pattern after passing through the transparent wing-sample. The laser powers are around 5–200 mW which is below their damage thresholds. The far-field diffraction pattern was observed for (i) broadband femtosecond laser pulses centered at 800 nm (top row in figure 2), (ii) a cw 532 nm green laser, and (iii) a cw 632 nm red laser (bottom row in figure 2). Note that the intensity profile of the diffraction pattern was recorded on a calibrated screen for all the cases. For nearly Gaussian input beams, the observed intensity pattern $I(x, y)$ exhibited a bright central spot and up to two distinct higher order maxima in the form of curved lobes. These lobes are symmetrically located on both sides of the bright central spot. The femtosecond pulse is used to show the robustness of the diffraction pattern under broadband coherent source in the IR range. We have computed the corresponding spatial frequencies along $x$ and $y$ axes, $k_x = (2\pi/\lambda D) x$ and $k_y = (2\pi/\lambda D) y$, respectively. Here D is the screen to wing distance and x, y are distances measured on the screen from the central spot. The position of the first lobe in the case of cw lasers corresponds to spatial frequency of around $0.5 \times 10^{-6}$ rad m$^{-1}$. These spatial frequencies agree well with our theoretical analysis, as shown later. The corresponding intensity-cuts of the diffraction patterns along x axis (right columns in figure 2) confirm these values.

To understand the formation of the diffraction pattern we performed SEM images of the wing surface (figure 3). The high resolution SEM images showed that the wing surface is decorated with a large number of non overlapping microstructures. The flat background of the wing exhibits nano-grain-like features which are typically smaller than the wavelength of the light. These microstructures were elongated having typical length and width around 5–8 $\mu$m and 1–2 $\mu$m, respectively. Note that their dimensions are comparable to the
used laser wavelengths, which act as efficient ‘photonic elements’ that diffract light.

To demonstrate how the observed far-field diffraction pattern emerges by a large number of microstructures (typically $10^4 \sim 6$ in beam waist of 1.0 mm) we computed Fast Fourier transforms (FFT) of the SEM images at various scales. The SEM images contained total $N \times M$ pixels and it was sampled with the spatial resolution $\Delta x = L/M$ and $\Delta y = L/N$ along $x$ and $y$ axes, respectively. The complementary Fourier domain then had spatial frequencies $k_x = m\Delta k_x$ and $k_y = n\Delta k_y$ where $m, n$ are integers and $\Delta k_x, y = 2\pi/L$ determines the resolution of the Fourier domain [20, 21]. As we increased the area of SEM image to incorporate an increased number of microstructures (figure 3) the corresponding FFT showed an emergence of the higher order lobes. The corresponding spatial frequency was around $0.5 \times 10^{-6}$ rad m$^{-1}$ that agrees quantitatively with the location of the experimental one. One can define a quasi-periodic function $A(x, y)$ that determines the average spacing between microstructures. As one can see in the SEM image, the quasi-period $A(x, y)$ is a function of position rather than a constant. Therefore, the input beam would be diffracted by an angle $A(x)\sin\Theta_x = m\lambda$ [22]. The corresponding average period between the microstructures along the $x$-axis was around 12 $\mu$m which also agrees well with the SEM image analysis.

To further prove that the diffraction lobes are due to quasi-periodic organization of microstructure array, we performed an experiment using a tightly focused laser beam. Using a high numerical aperture (NA 0.2) triplet-lens objective we generated a micro-spot of full width <5 $\mu$m. Note that this spot size is comparable with the typical size of a single microstructure. We recorded the diffraction pattern on a screen kept about 5 cm away for two different cases when the laser spot is on the microstructure and when the laser spot is on the background, i.e. between the two microstructures. We observed that these diffraction patterns are qualitatively different compared to the case where we used a 1 mm collimated beam. The FFT of high resolution SEM image with single microstructure also produced a similar complex diffraction pattern that matched with the experimental observations (see figures 3 and 4). This demonstrated that the observed diffraction is a result of quasi-periodicity in the array of microstructures. In the following we develop a theoretical model to explain our experimental results.

Figure 3. Left column: SEM images of the wing surface (25 nm gold coated) at varying length scales (a) 200 $\mu$m, (d) 100 $\mu$m, (g) 10 $\mu$m, (j) 1 $\mu$m. Middle column: computed FFT pattern shown as (b), (e), (h), (k) for the corresponding images on its left. Right column: computed intensity cut for the corresponding FFT image.
3.2. Theoretical understanding of the experimental results

We present a simple quantitative model to provide further insight into the experiment by generating the far-field diffraction from a 2D array of non-overlapping microstructures. Each micro-structure was modeled by an amplitude transmission function $t(x, y)$. The total transmission $T$ of the wing was then due to $N \times M$ total number of microstructures arranged in a lattice with average distance between them being $d$. A similar jitter model was previously proposed to explain spectral properties of an array of identical non-overlapping grains [32–38]. However, we introduce multiple disorders to model the laser diffraction through complex wing surface.

We generated a 2D total brightness distribution $T(x,y)$ by creating square cells, each containing one microstructure, arranged in a rectangular array of size $N \times M$. The distance between two microstructures in the square lattice was $d$ along the two orthogonal directions. In the case of perfectly ordered array of identical microstructure, one can write,

$$T(x,y) = t(x,y) \star \sum_{n=0}^{N-1} \sum_{m=0}^{M-1} \delta (x - nd) \delta (y - md),$$  \hspace{1cm} (1)

where the star denotes convolution and $\delta(x)$ is a Dirac delta function centered at $x = 0$.

To model structural organization of microstructures on the wing surface, we added disorder in the shape of each microstructure, their position and orientation. The resulting stochastic 2D brightness function is given by,

$$T(x,y) = \sum_{n=0}^{N-1} \sum_{m=0}^{M-1} t(R - \alpha_{m,n} - \xi(n) - \eta_{m,n}),$$  \hspace{1cm} (2)

where $\alpha_{m,n}$ is the position vector of each microstructure, $\xi$ defines a two component stochastic vector quantifying the deviation of their position from their nominal center and $\eta_{m,n}$ is a set of variables determining the shape distortions. The stochastic variables are delta correlated in position as,

$$\langle \xi_{x,y} (n) \xi_{x',y'} (n') \rangle = A_{x,y} \delta (n - n'),$$  \hspace{1cm} (3)

where $A_{x,y}$ denote the amplitude of the noise along x and y directions.

The Fourier transform of the transmission is given by $T(k_x, k_y) = \text{FT}(T(x,y))$ where $k_{x,y}$ denote spatial frequencies [39]. The average normalized far-field diffraction pattern is given by,

$$I(x,y) = C |T(k_x, k_y)|^2$$  \hspace{1cm} (4)

where $C$ is a proportionality constant. Note that the above equation is usually used for monochromatic incident field and it can be extended to a broadband pulse of width $\Delta \lambda$ that could lead to further detail of quasi-periodicity [39, 40].

We simulated the above model in Matlab by generating the transmission function $t(x, y)$ in the form of a curved ellipse of about 2 $\mu$m width and about 8 $\mu$m length which is comparable to the dimensions observed in the SEM image. Each micro-structure consisted of 16 $\times$ 16 pixels where each pixel defined 750 nm resolution. The total number of microstructures in our simulations were more than $10^3$. We verified that in the case of a perfect square grid of identical microstructures (equation (1)), the simulated diffraction pattern consisted of ordered spots typical of regular diffraction grating (figures 5(a) and (d)).

To model our experiment we generated complex aperture functions by adding various kinds of disorders in the shape,
orientation and position of each element. In figure 5(b), we show the disordered array which is produced by adding noise on the shape and orientation of \( t(x, y) \). The variance in the fluctuations in the length and width was \( 8 \pm 4.5 \) \( \mu m \) and \( 2 \pm 0.75 \) \( \mu m \), respectively and their orientation was allowed to fluctuate randomly by \( \pm 30^\circ \). The chosen parameters lie in the range seen in the SEM images. The corresponding far-field diffraction pattern for this organization showed that higher than second order spots vanished but their organization was on the square grid.

Finally, to create the various symmetries in the form of rings, we randomly selected several patches and added a global rotation of less than \( \pm 15^\circ \) in order to create a wavy pattern on the grid structure. In this case, various symmetries overlapped and produced a ring-like pattern similar to the experiment (see figure 5(c)). Note that the location of the first order peak corresponds to spatial frequency \( 0.5 \times 10^{-6} \) rad m\(^{-1} \) close to the ones seen in the experiment as well as in the Fourier analysis of the SEM images. Although the minimally stochastic model uses many approximations, it can reproduce key signatures of the experiment quite well.

4. Signature of symmetry: rotations of the diffraction pattern at various length scales

We can probe local variations in the structural arrangement across the wing by simply scanning the laser beam. This is possible since the 1 mm beam spot was much smaller than the wing size >1 cm. To demonstrate this, we recorded the diffraction pattern at various scales by scanning the laser spot along the wing length (figure 6). A rotation of the original diffraction pattern was observed for both the pulsed and cw lasers. This behavior directly reflects the local symmetry and its spatial correlation along the entire wing surface. It should be mentioned that one can easily vary the spot size to further probe local and the global organizations in a single-shot manner. The observed rotation in the diffraction pattern suggests a systematic rotation in the arrangement of the hooks without much change in their density and shape.

To verify this, we recorded SEM images of various portions of the wing and numerically computed FFT of the images (figures 6(k)–(o)). The SEM-FFT analysis also produced a similar rotation in the diffraction as shown in figure 6. This confirmed our optical observation. Therefore, our technique is very attractive and efficient to reveal the complex arrangements of millions of these photonic elements on the wing surface. We have observed similar results in insect wings of the Drosophila which suggest a generic nature of the reported phenomenon. The functional significance of these rotations and their developmental aspects requires further experimentation.

5. Observation of genetic mutation in the photonic architecture through diffraction pattern

We report the first optical measurements of reorganization in microstructure array due to the genetic mutations on the transparent wings of the Drosophila melanogaster. The Drosophila wings are an ideal system for this kind of study since the development, structure and function of the wings have been studied extensively and the roles of several genetic mutants are well characterized. For our analyses we selected two different mutants, Cyo and vg, that either produce curly wings or generate small stumpy wing rudiments, respectively. The wild-type unmutated wings were kept as controls (reference). Although the developmental dynamics of Drosophila wings along with the implications of these mutants on the structural
and functional aspects have been explored, much less is known about how these mutations affect the organization of the microstructures on the wing surface. Using diffraction pattern in transmission offers us a unique optical technique to make a quantitative comparison among wings produced by various mutations.

First, we recorded the diffraction pattern from a normal fly-wing as our control. This clearly showed a ring-shaped pattern revealing average periodicity of around 20 $\mu$m. Compared to the control wing, in Cyo wings, the diffraction ring became smaller with the appearance of a weak second order lobe. This suggests that the average periodicity increased and their organization became more ordered compared to the normal wings. In contrast, in vg wings the diffraction pattern was speckle-like without any higher order maxima and minima. This means that in this case both the symmetry and average periodicity are completely absent. Note that if such information is attempted by SEM imaging, it would be a very tedious and inefficient process. This is clearly illustrated in figure 7 where SEM images of various mutants are recorded and corresponding FFT is computed. The good matching between computational and experimental patterns again confirmed the implications of this technique. The spatial coherence of the laser and sensitivity of the diffraction pattern offer a unique advantage over other methods. This experimental evidence of global correlation and its genetic control could be potentially useful to understand how one can manipulate genes to control the natural photonic architecture as well as for other potential biological applications in understanding structure-function relation in the genetic pathways.

6. Conclusion and outlook

In summary, we show that the diffraction pattern through transparent insect wings is correlated with the spatial organization of the microstructures at various length scales. We demonstrate that the microstructures on the transparent wings possess a long-range quasi-periodic order and characteristic organizational symmetry as unveiled by an appearance of the stable and robust diffraction pattern. These observations are in quantitative agreement with a Fourier analysis of high resolution SEM images of the wing surface. The existence of average periodicity was supported by observations of diffraction pattern of single microstructure and background using a tightly focused laser beam. Furthermore, we proposed a simple quantitative model to explain our observations that showed the existence of minimal disorder in the microstructure organization. Two different applications of our optical technique were demonstrated. First, by scanning the laser beam across the wing surface, a rotation of the original diffraction pattern was observed that demonstrated symmetry in the spatial organization of microstructures. Second, we reported first optical measurements on how various genetic mutations reorganize the biophotonic architecture.

Figure 6. Rotation of the original diffraction profiles by scanning the laser beam across the wing sample. Top row (a)–(e): experimental observations using broadband femtosecond laser; middle row (f)–(l): with a green cw laser; (k)–(o): theoretical rotation pattern produced by computing FFT of SEM images at different areas of the wing shown below (p)–(t).
The proposed optical technique is potentially attractive to quantify natural photonic architecture on a large variety of transparent insect wings in a single-shot manner. These tools would be crucial to understand design principles of photonic crystals with potential for biomimetic applications that may lead to novel optical devices [2, 18, 41].

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Figure 7. Reorganization of photonic microstructures by genetic mutations. Left column top to bottom shows the SEM images of the wing for wild-type unmutated wing, Cyo wing mutation, and vg mutation, respectively. Middle column: FFT of the SEM image for corresponding SEM image. Right column shows the observation of the diffraction pattern with a green laser for the corresponding mutations.
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