Application of AFM Measurement and Fractal Analysis to Study the Surface of Natural Optical Structures

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Abstract. *The wings scales of the butterflies were studied by Atomic Force Microscopy (AFM) in the air. Measurements were done without special preparation of species in order to observe the surface in real conditions. The data of probe microscopy (figures) confirm AFM to be a powerful technique for determining features of the insects' wings. These features play a key role in optical phenomena which makes fascinating wings coloration. The structure determines light reflection, propagation, and diffraction. AFM imaging was done at the areas of specific colors without scale separation.*

Keywords

Atomic force microscopy, diffraction grating, fractal analysis, structural coloration, wing surface.

1. Introduction

The natural structures are sources of inspiration for design of artificial devices for many years. One of such structures could be found at the wings of the butterflies and moths [1]. The exceptional optical and mechanical properties make them important for a number of applications [2], [3] and [4]. The fractal concept is widely used in biological sciences to characterize the irregular complex structures [5], [6] and [7]. On the other hand, fractal geometry offers new and valuable opportunities to describe and compare complex individual or species-specific patterns. It provides an integrative measure that captures the complexity of a whole pattern when explored at different scales, which would be a great help to study their variability and functionality [8] and [9]. Fractal analysis describes the geometrical complexity in the wings of several, taxonomically different butterflies, in terms of their fractal dimension. It was used in several studies from biological literature [10]. Two groups of butterflies were chosen for this study. Morphology and surface structure of the wings scales were investigated. First is Euploea mulciber, known as "Striped Blue Crow", and the second is Morpho didius, also named as "Giant Blue Morpho". Both species exhibit strongly angle dependent coloration of wings. Our analysis was carried out using 10 specimens for each species. The darkest and the brightest areas of wings were studied. Even ordinary optical microscopy can show that the scales are different along the wing surface; it depends on the distance from the body. Some of them are even modified into the tiny tubes. Forewings of the butterflies were studied at discal and postdicsal areas. The choice of areas could be explained by differences in color and consequently the surface structure (Fig. 1): the one that reflects light and looks shiny, and the other looking dark and lusterless.

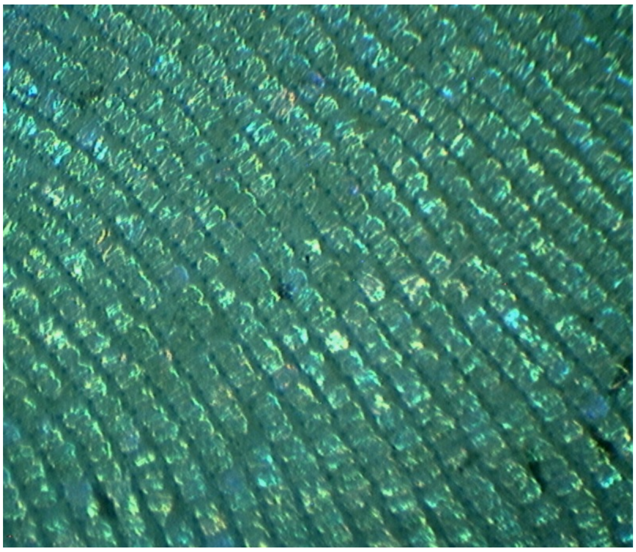


Fig. 1: Rows of scales, the iridescence is created by physical phenomena.

2. Experimental

2.1. Atomic Force Microscopy

AFM (Atomic Force Microscope) NTEGRA (NT-MDT production) was used to study the surface topography in semi-contact mode. No special preparation of the sample was done: just cutting of a piece of wing by scissors and fixing it by a tape on the substrate from the bottom side of the wing. All measurements were performed in the same laboratory, at room temperature (296 ± 1 K) and 50 ± 1 % relative humidity. The measurements were repeated three times for each sample on different reference areas, to validate the reproducibility of the data. Statistical analyses were performed using the GraphPad InStat version 3.20 computer software package (GraphPad, San Diego, CA, USA) [11]. The data of AFM represent only the surface appearance, without explanation of inner structure of the wings. The scale surface ridges are responsible for direction of light wave's propagation. These structures define the part of spectrum which is absorbed or penetrates to the next bottom layers. The upper topography texture of the wings represents diffraction grating (Fig. 2).

Smaller scanning area allows detailed observation of the surface topography (Fig. 3).

The native software of the microscope provides processing of the results. Figure 4 demonstrates High-High correlation graphs of both species. It characterizes lateral distribution of surface features - the distance of features' high correlation. The distance between surface features as well as their shape forms the diffraction grating on the surface and contributes the scale color.

The characteristic wavelengths of the spatial periodicity are measured using a spatial power spectrum (Tab. 1). The wavelength periodicity also shows the difference between species and different colors on the same wing. There is one large peak (sharp at a particular wavelength) on each graph and smaller peaks surrounding (Fig. 5).

2.2. Fractal Analysis

Cube counting method, based on the linear interpolation, applied for AFM data, was used for fractal analysis of the butterflies' wings, which is described in detail in [12]. Cube counting method [12] is derived directly from a definition of box-counting fractal dimension. The algorithm is based on the following steps: a cubic lattice with lattice constant l is superimposed on the z-expanded surface. Initially l is set at $X = 2$ (where X is length of edge of the surface), resulting in a lattice of $2 \times 2 \times 2 = 8$ cubes. Then $N(l)$ is the number of all cubes that contain at least one pixel of the image. The lattice constant l is then reduced stepwise by factor of 2 and the process is repeated until l equals to the distance between two adjacent pixels. The slope of a plot of log $N(l)$ versus $\log 1 = l$ gives the fractal dimension D directly. The results of the fractal dimensions (D) for AFM images of wings areas of all samples are shown in Fig. 6.

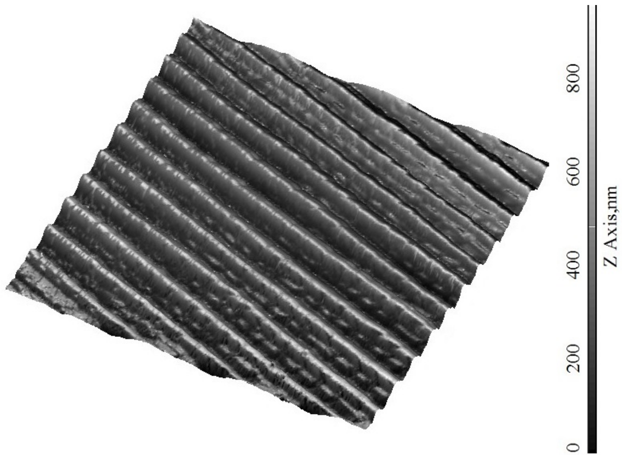
The results of the fractal dimensions (D) with coefficients of correlation (R_2) are given in Tab. 2. For all analyzed cases (Tab. 2), the coefficients of correlation (R_2) associated with fractal dimensions D were greater than 0.99 representing a good linear correlation. An (R_2) of 1.0 indicates that the regression line perfectly fits the data.

The texture of wings scales is semiregular. It is also found that the geometrical complexity of the butterflies' wings shows clear distinctions for the two groups of species in terms of their fractal dimension. These results provide us additional methods for distinguishing species and their distinctive colors (differences in intensity and tonality).

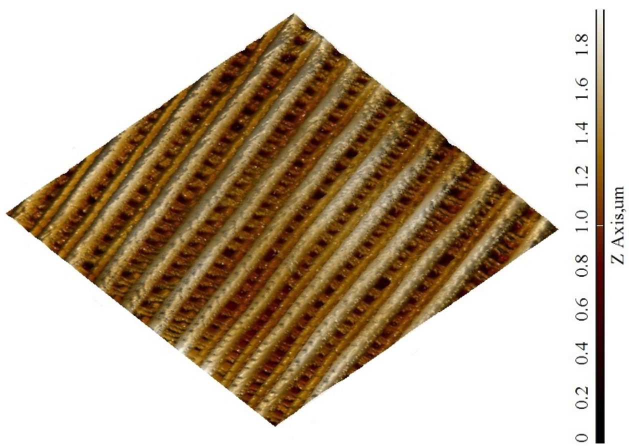
2.3. Thermocamera Imaging

Since AFM is limited by height of the sample, the imaging was carried out on flat scales of the wings (not on veins of wing construction). But these construction elements are well seen on thermocamera images. Thermocamera imaging mostly depends on the material nature and its inner structure. It partly represents macrostructure of the wing.

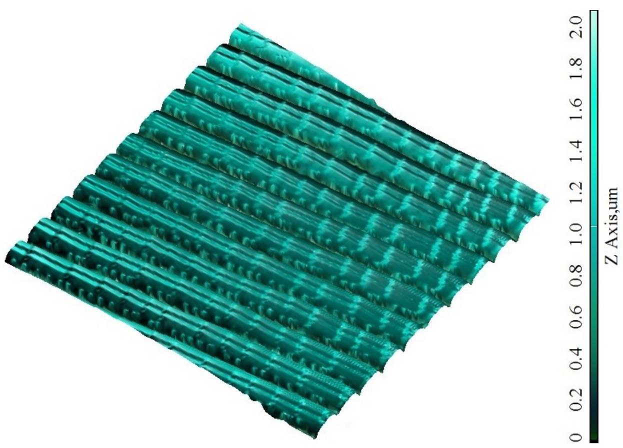
The thermocamera was used to follow the behavior of wings in relation to heated objects: the butterfly



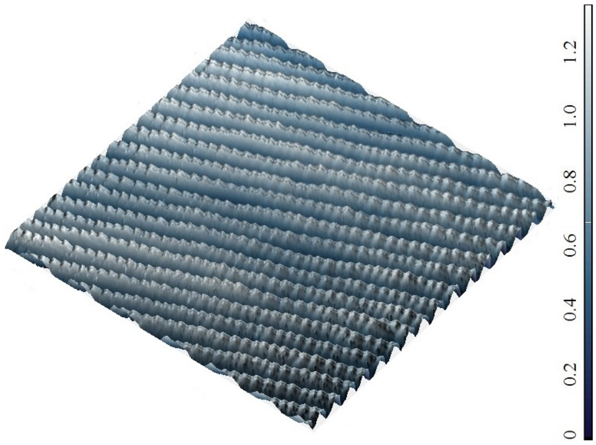
(a) Giant blue morpho samples (black color).



(b) Striped blue crow samples (brown color).

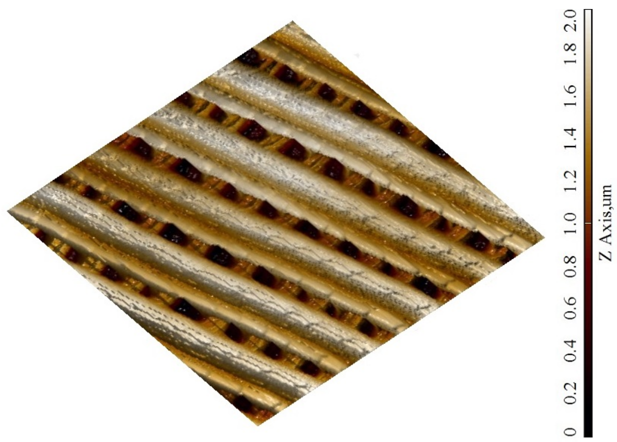


(c) Giant blue morpho samples (blue color).

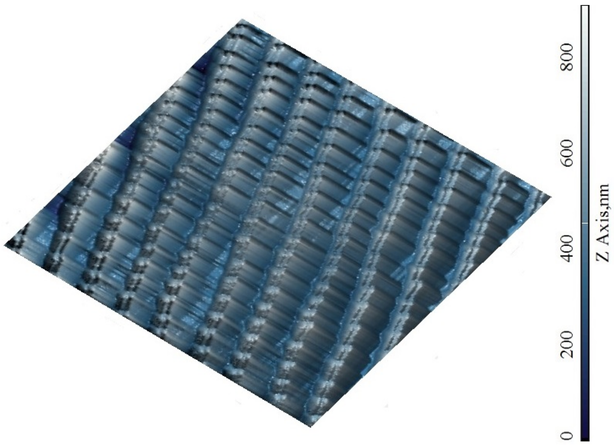


(d) Striped blue crow samples (blue color).

Fig. 2: AFM images of wing area (area scan 20×20 µm).



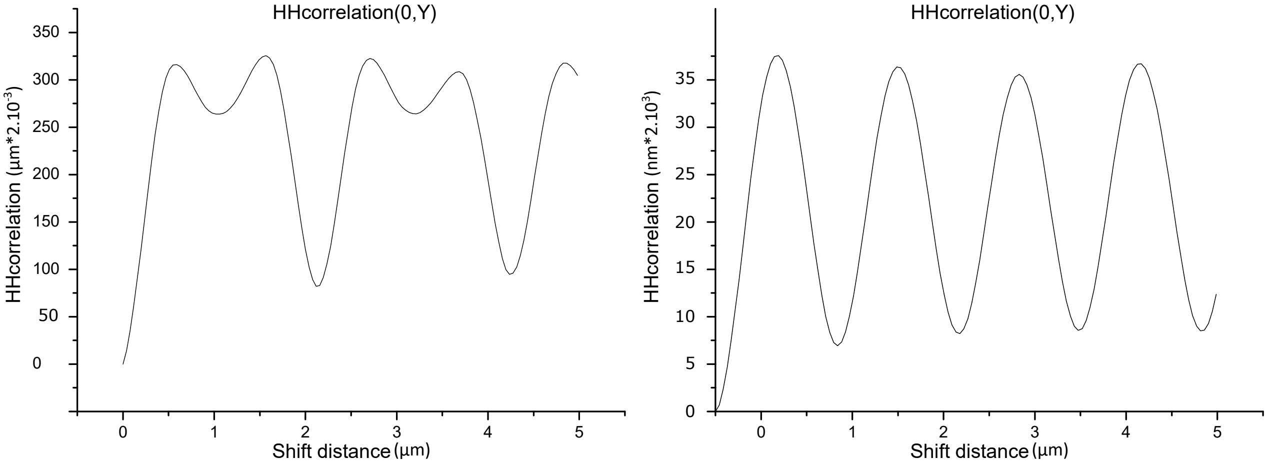
(a) Brown color.



(b) Blue color.

Fig. 3: AFM images of wings area of striped blue crow samples (area scan 10×10 µm).

wings seem to be transparent. The heated pattern is well observed through the wing in the image from camera (Fig. 7). The transparence helps butterflies to be invisible for most of predators. It is well known that a number of predators have very good vision in IR spectrum. Only the veins show the

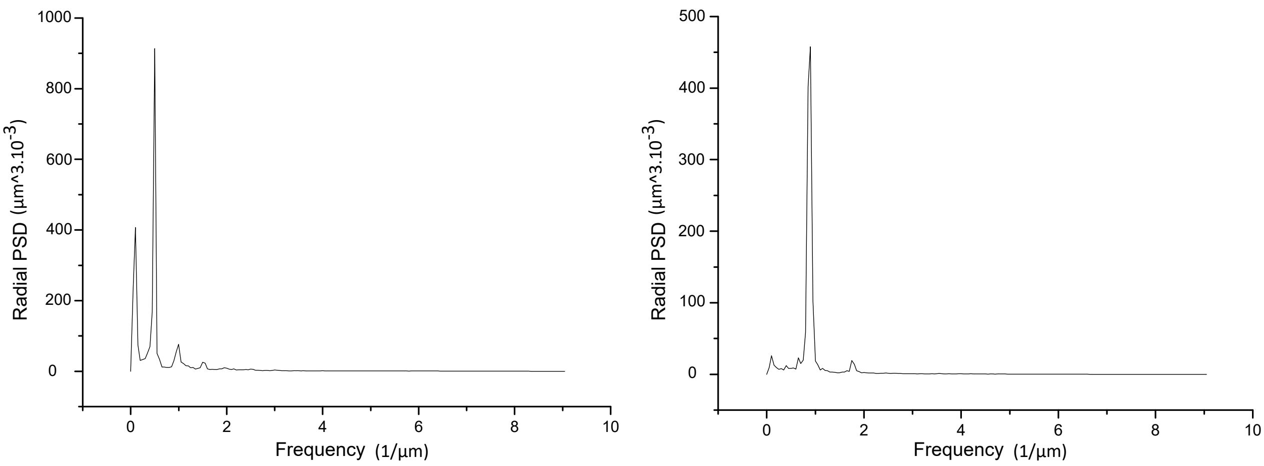


(a) Brown color. (b) Blue color.

Fig. 4: High-High correlation graphs that correspond to Striped Blue Crow samples.

Tab. 1: Radial wavelength and radial wavelength index for the two groups of butterflies.

Parameters	**Striped Blue Crow samples (brown color)**	**Striped Blue Crow samples (blue color)**	**Giant Blue Morpho samples (black color)**	**Giant Blue Morpho samples (blue color)**
Radial Wavelength (µm)	10.0060	9.9990	1.6660	19.9990
Radial Wavelength Index	0.0616	0.0995	0.0794	0.0686

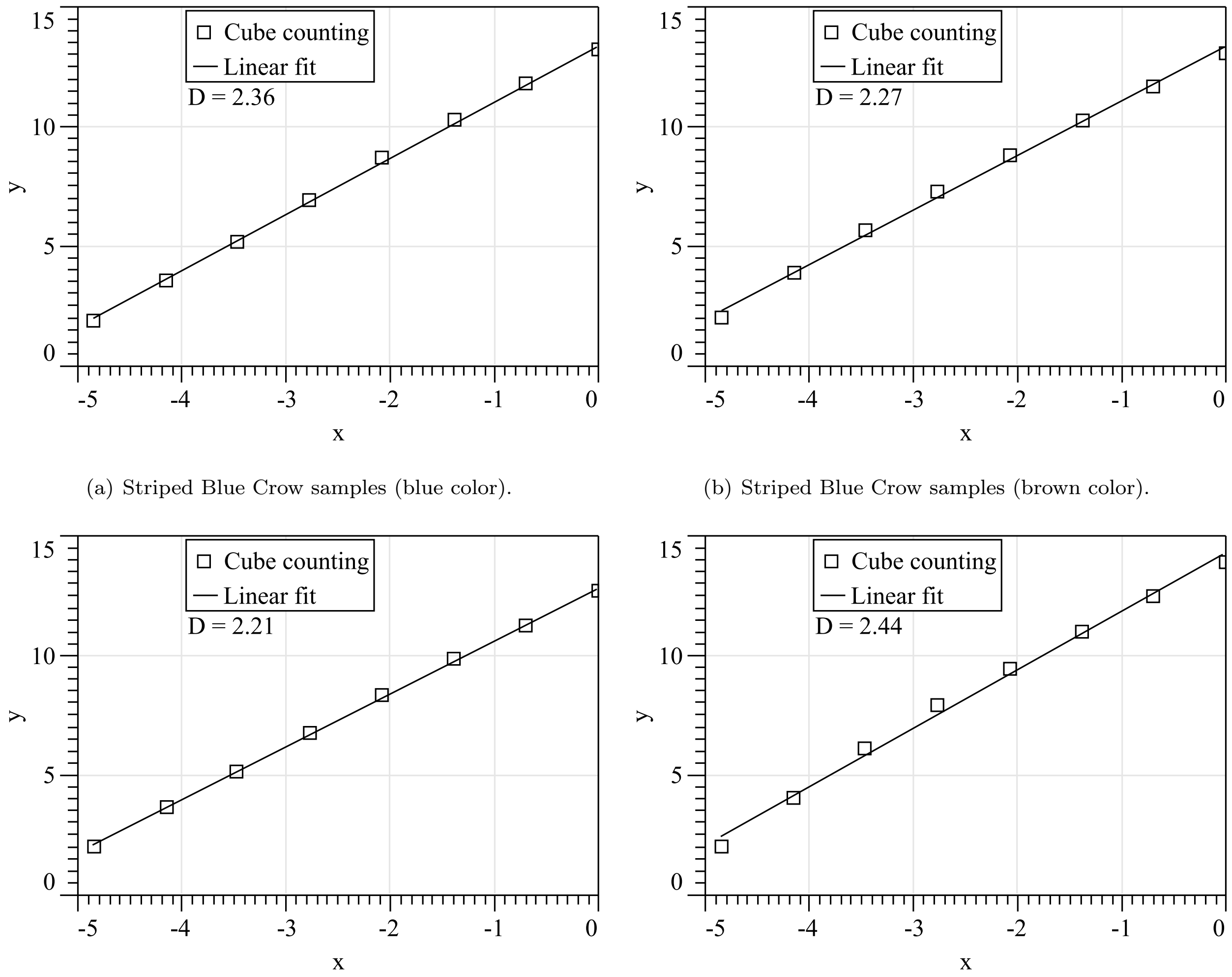


(a) Brown color. (b) Blue color.

Fig. 5: Radial power spectrum density graphs that correspond to Striped Blue Crow samples.

presence of the wings between thermocamera and heated object. This could be considered as one more protected mechanism for surviving (besides imitation of tree and grass leaves or eye spots on the wings).

The basic properties of the height values distribution of the surface samples (including its variance, skewness, and kurtosis), computed according to [12] are shown in Tab. 3.



(a) Striped Blue Crow samples (blue color).

(b) Striped Blue Crow samples (brown color).

(c) Giant Blue Morpho samples (black color).

(d) Giant Blue Morpho samples (blue color).

Fig. 6: Fractal dimension for AFM images of wings areas (area scan 20×20 µm).

Tab. 2: The fractal dimensions (D) with coefficients of correlation (R^2) determined by the cube counting method, based on the linear interpolation type, of the two groups of butterflies: a) Striped Blue Crow sample; b) Giant Blue Morpho sample. Scanning square areas of 20×20 µm.

Parameters	**Striped blue crow samples (brown color)**	**Striped blue crow samples (blue color)**	**Giant blue morpho samples (black color)**	**Giant blue morpho samples (blue color)**
D	2.27 ± 0.02	2.36 ± 0.02	2.21 ± 0.018	2.44 ± 0.018
R^2	0.995	0.995	0.996	0.996

3. Discussion

A lot of natural objects have a fractal structure. The structure is repeated at the lower metric range. The wing scales are fractal photonic structures which are developed by nature. It is one of the ways to manipulate the energy of sun. In order to analyze the influence of a structure on coloration, we scanned different colors area of two species. Branches of micro and nano-sized features form the wing scale [13]. Although the diffraction grating is observed at all cases, there are differences in correlation lengths and fractal data of the surfaces. Diffraction grating of E. mulciber specie was studied by F. Mika in [14] by SEM. The advantages of AFM in this case before SEM are measurements in real conditions (air, humidity) and obtain real 3D data about surfaces topography. Correlation lengths (Lx = 0.882 µm and Ly = 0.352 µm for blue color, and Lx = 0.800 µm Ly = 299 µm for brown color of Striped Blue Crow wing sample) are the characteristic lengths over the scanned surface. Correlation length depends of roughness of the surface: the higher roughness, the smaller correlation length [15]. Consid-

Tab. 3: The basic properties of the height values distribution (including its variance, skewness and kurtosis) of the two groups of butterflies: a) Striped Blue Crow sample; b) Giant Blue Morpho sample. Scanning square areas of 20×20 µm.

The basic properties of the height values distribution of the surface samples	Striped blue crow samples (brown color)	Striped blue crow samples (blue color)	Giant blue morpho samples (black color)	Giant blue morpho samples (blue color)
	Values	Values	Values	Values
Ra (Sa) (µm)	0.439	0.66	0.816	0.351
Rms (Sq) (µm)	0.543	0.828	0.933	0.456
Skew (Ssk) (-)	-0.279	0.755	-0.106	-1.35
Kurtosis (Sku) (-)	-0.07	0.248	-1.14	0.981
Inclination θ (°)	3.2	5.7	4.7	5.2
Inclination φ (°)	-0.2	7.4	32.7	-173.6

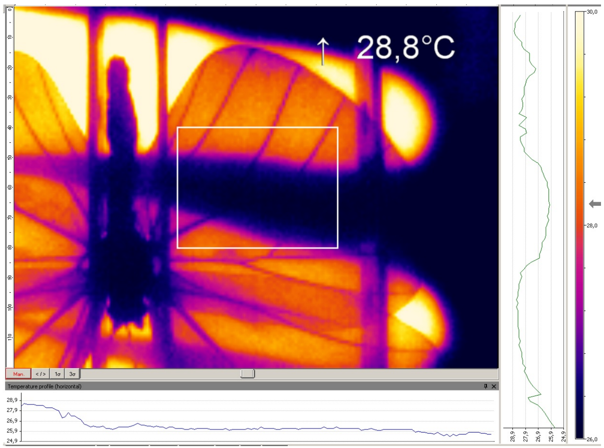


Fig. 7: Thermocamera image of fingers behind the wings.

ering this, the attention is paid to the quality of the AFM measurements, since the noise is close to zero correlation length [15]. The data for fractal analysis were measured with the same magnification. Fractal dimensions correlate with the scales morphology. So, black and brown color surfaces have lower fractal dimension than the blue areas. And consequently, these values relate to reflectance of the surface [16], [17], [18], [19] and [20]. Multilayered structure of the scales and their arrangement contribute the colors of wings. However, surface features also play important role (which are diffraction gratings for visible light). They are well seen in AFM images [21], [22] and [23]. The chitin elements have different shapes: the oriented layers on the ridges and well visible notches constriction. The crossribes between ridges form a complex three-dimensional structure. The variation of surface structure provides obtaining the colors from iridescent to antiglare.

4. Conclusion

There are many optical systems created by nature. One of them is the wing scale which has superior optical and hydrophobic surface properties. It was found that different color areas have different three-dimensional (3D) structure of the surface. The 3D structure represents complicated semi-ordered combination of surface features. The sizes of these features are comparable to visible light wavelengths. The studied 3D topography is a first step in color producing: the diffraction grating of the scale surface defines further propagation, transmission and reflection of light. The wings seem to be transparent at IR radiation since it is possible to see heated objects through the wings by thermo-camera. Here, by combining correlational and experimental evidence, we also describe the surface of scales as physical structures, which have a fractal nature and can reveal biologically meaningful information. The ridges of the scale surface have fractal properties and are oriented in one direction. The morphology of the analyzed samples provides additional description about the structural features of the butterflies' wings 3D surface topography. Both theory analysis and experimental results suggest that AFM, the statistical and fractal analysis can provide additional insight into the wings 3D morphology and can be included in an algorithmic mathematical model.

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Appendix

The basic properties of the height values distribution, including its variance, skewness and kurtosis, computed according the Ref. [12] is defined as follows:

- RMS value of the height irregularities: this quantity is computed from data variance.
- *Ra* value of the height irregularities: this quantity is similar to RMS value with the only difference in exponent (power) within the data variance sum. As for the RMS this exponent is $q = 2$, the *Ra* value is computed with exponent $q = 1$ and absolute values of the data (zero mean).
- Height distribution skewness: computed from 3rd central moment of data values.
- Height distribution kurtosis: computed from 4th central moment of data values.
- Mean inclination of facets in area: computed by averaging normalized facet direction vectors.
- Variation, which is calculated as the integral of the absolute value of the local gradient.