Investigation and application of photonic nanoarchitectures of biological origin occurring in butterfly wing scales

PhD thesis booklet

Gábor Piszter

Supervisors: Prof. László Péter Biró, corresponding member of the HAS

HAS Centre for Energy Research Institute of Technical Physics and Materials Science

Dr. Krisztián Kertész

HAS Centre for Energy Research Institute of Technical Physics and Materials Science

Consultant: Dr. Attila Barócsi

Budapest University of Technology and Economics Faculty of Science

Budapest

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Introduction and motivation

Colors in nature, especially those produced by living organisms, often have unique properties. By using colors, living beings can adapt to their environment, which is why they have developed coloration for various tasks during the millions of years of evolution. With the use of colors, individuals can camouflage, warn or deceive potential predators [Ruxton, 2004], and they can act as a sexual communication signal [Kemp, 2007].

Colors in the animal kingdom can be produced in two ways. The so-called chemical colors always originate from pigments or dyes which are capable of selective molecular absorption of light, thus reflecting only certain ranges of the visible spectrum. In the other, "physical" way of color generation, the light is reflected from the surface of photonic nanoarchitecture, because certain wavelength ranges cannot propagate in this structure [Biró, 2011]. Structural colors often have particular optical properties. Both the hue and reflected intensity may vary depending on the direction of the illumination and detection, therefore many variations can be observed from the dull, single-colored surface [Kertész, 2006] through metallic shine [Plattner, 2004] to iridescent surfaces [Yoshioka, 2007].

In the last decades, from the point of view of physics and materials science, the most important tasks were investigation of structural colors, and the understanding of the mechanism of color-generating photonic nanoarchitectures. Hence, the knowledge gained inspired the production of artificial, "bioinspired" nanoarchitectures [Zhao, 2012] and later potential applications also emerged [Potyrailo, 2013]. On the other hand, it is also important to examine the biological background of structural colors. For example, the investigation of biological functions relevant to individuals and the whole population, as the role of structural colors in the life of butterflies, and the natural diversity within a population can provide information about the behavior and the formation of these animals, and about the details of the development of structural colors. The discovered relationship can also be effectively utilized in applications that use biological samples.

Objectives

In this work, I studied the photonic nanoarchitectures on the wings of blue male polyommatine butterflies using materials science methods (optical microscopy, scanning and transmission electron microscopy), and the properties of the generated structural colors using various optical spectrometric techniques. I explored the relationship between the physical properties and the biological functions and used the gained knowledge in the development of a vapor sensor device based on butterfly wing.

In the first part of the results, I investigated the species specificity of the structural colors and photonic nanoarchitectures of nine closely related polyommatine butterfly species living in the same habitat and I explored the relationship between the hues of the wings and the distribution of the imagines of these species within a year.

In the second part I studied the natural variability of the structural coloration of two similarly colored butterfly species living in the same habitat but differing in the mating strategy. I also investigated how the blue color of the dorsal surface and the ventral wing patterns could be modified by the controlled cooling of the *Polyommatus icarus* individuals in pupal stage.

In the third part, the photonic nanoarchitectures in the wing scales of *Polyommatus icarus* males were used as an optical vapor sensor. I showed that the sensor material enables chemically selective sensing, and by the modification of the surface of the photonic nanoarchitecture the vapor sensing properties could be tuned.

Experimental methods

In the dissertation I examined the optical and structural properties of samples of biological origin using various materials science techniques. The photonic nanoarchitectures in the wing scales of the butterflies are nanocomposites typically periodic on the hundred-nanometer scale, therefore the investigation of their structure requires higher resolution techniques than the optical microscopy. Scanning electron microscopy (SEM) provides detailed images of the surface of the photonic nanoarchitecture, while from the transmission electron microscope (TEM) images of the cross-sectional samples the three-dimensional configuration of the structure can be studied. I performed structural analysis of SEM and TEM images from the wing scales using the Biophot Analyzer software developed by the Nanostructures Department and the resulting data was further processed using an artificial neural network (ANN) based evaluation method.

The structural color and the iridescence of the wing scales can be examined either by naked eyes or using optical microscopy. Quantitative characterization of the wing colors is best achieved by the reflected spectra measured in the visible and near UV wavelength range of light. During my measurements I examined the wavelength distribution of light reflected from the wings using a modular spectrophotometer with different optical setups. Using the same instrumentation, I also examined the color changes of the butterfly wings during vapor exposition. The data obtained during the spectral measurements were analyzed using the visual color space of the polyommatine butterflies. The results were verified using principal component analysis-based evaluation and I showed that in the examined wavelength range the best separation of the data was achieved. Using the artificial neural network-based evaluation of the structural colors of the polyommatine butterflies I showed that, similarly to the structural properties of the photonic nanoarchitectures, they are species-specific.

New scientific results

1.A. In the case of polyommatine butterflies living in the vicinity of Normafa, I examined the optical and structural properties of the structural blue color and the color generating photonic nanoarchitectures of the dorsal wing surface of the male specimens. I showed for the first time that the structural blue color of all nine investigated species is species-specific, which means that based on the reflectance spectra the species can be identified with high precision using artificial neural network-based evaluation.

1.B. Using the color vision of the polyommatine butterflies I created for the first time the three-dimensional color space of these animals representing the data measured using the reflection spectra of the normal incident light on the dorsal side of the wings. I showed that the chromaticity points corresponding to the same species were clustered while the different species were separated. Based on the flight period histogram of the butterfly population around Normafa, I showed that imagines of similarly colored species are shifted in time in their habitat so that visual sexual communication can be effectively fulfilled.

1.C. Based on scanning and transmission electron microscopy I described for the first time the photonic nanoarchitectures of the nine species in detail. With artificial neural network evaluation, I showed that the photonic nanoarchitectures that generate the structural color are also species-specific, meaning that the species of the butterflies can be precisely identified based on the structural parameters measured on the microscope images of the wing scales.

These results are published in: [T1], [T2]

2.A. I showed that the structural colors of *Polyommatus icarus* and *Plebejus argus* are species-specific. I compared the deviation properties of the data measured with perpendicular incident light reflectance and integrating sphere and showed that the latter optical measurement setup is more suitable for investigating intra-species color variability. For the four wings of 25-25 individuals from each species (100-100 samples) I showed it for the first time, that the small intra-specific variation of the structural color does not depend on the species of the butterfly, or on their mating strategy, which is attributable to the use of the structural color as a sexual communication signal. I showed, that the brightness of the structural color of the two species is related to their mating strategy: the color of the patrolling species is more intense than the color of the lekking species.

2.B. I showed that it is possible to change the physical characteristics of the imagines of *Polyommatus icarus* by the long-term cooling of pupae. Cooling the home-grown pupae of the butterflies from ten days to more than two month I showed that the pigment-based colors of the ventral wing pattern change in proportion with the cooling time. In contrast, the spectral properties of the structural color were only slightly dependent on the cooling time, the presence of individual variation was much more pronounced, which can be explained by the hidden genetic variations of the species.

These results are published in: [T3], [T4]

3.A. I showed that the structural color of the polyommatine butterflies changed in a reversible and reproducible way when the atmosphere surrounding the wings was replaced by a mixture of volatile vapor and air. I developed a measurement protocol and a data processing software demonstrating that the color change of the butterfly wing-based sensor is proportional to the ethanol vapor concentration.

3.B. Using the three-dimensional color space of the butterflies I showed, that using seven test volatile vapors the blue color of the male *Polyommatus icarus* butterflies changes in a material-specific way, meaning that the wing acts as a chemically selective sensor. By verifying the result using principal component analysis I showed that the color space of the butterflies provides optimal separation of the data, and therefore it is a suitable tool for evaluating the vapor sensing measurement data.

3.C. I showed that the governing process of vapor sensing is capillary condensation in the low concentration range, while in the case of high concentrations the swelling of the chitin nanoarchitecture is dominant. The photonic nanoarchitecture in the wing scales was coated using 5 nm thick Al_2O_3 layer. By isolating the chitin from the vapors, I demonstrated the color change resulting from the swelling of the photonic nanoarchitecture is eliminated, causing the degradation of both sensitivity and chemical selectivity.

3.D. By soaking the butterfly wings in various volatiles, I showed that this pretreatment also affects the chemical selectivity and sensitivity. The 14-day-long soaking of the butterfly wings in ethanol increased both the chemical selectivity and the intensity of the spectral response.

These results are published in: [T5], [T6]

Publications related to thesis statements

T1 **Piszter G.**, Kertész K., Vértesy Z., Bálint Zs., Biró L. P.: Color based discrimination of chitin–air nanocomposites in butterfly scales and their role in conspecific recognition. *Analytical Methods* **3** (2011) 78–81.

The work also appeared on the title page of the journal:

T2 Bálint Zs., Kertész K., **Piszter G.**, Vértesy Z., and Biró L. P.: The well-tuned Blues: the role of structural colours as optical signals in the species recognition of a local butterfly fauna (Lepidoptera: Lycaenidae: Polyommatinae). *Journal of the Royal Society Interface* **9** (2012) 1745–1756.

T3 **Piszter G.**, Kertész K., Bálint Zs., Biró L. P.: Variability of the structural coloration in two butterfly species with different prezygotic mating strategies. *PLoS ONE* **11** (2016) e0165857.

T4 Kertész K., **Piszter G.**, Horváth Z. E., Bálint Zs., Biró L. P.: Changes in structural and pigmentary colours in response to cold stress in *Polyommatus icarus* butterflies. *Scientific Reports* **7** (2017) 1118.

T5 **Piszter G.**, Kertész K., Vértesy Z., Bálint Zs., and Biró L. P.: Substance specific chemical sensing with pristine and modified photonic nanoarchitectures occurring in blue butterfly wing scales. *Optics Express* **22** (2014) 22649–22660.

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12 **Piszter G.**, Kertész K., Bálint Zs., Biró L. P.: Matematikai pontossággal látnak a lepkék. *Természet Világa* **146** (2015) 112–115.

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