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FULL ARTICLE

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Naturally safe: Cellular noise for document security

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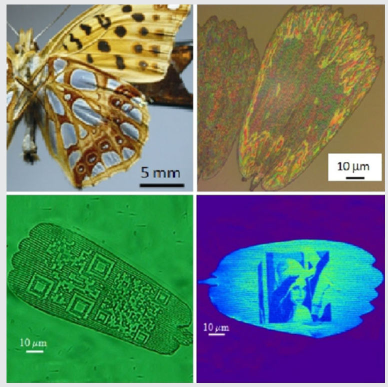
Abstract

Modern document protection relies on the simultaneous combination of many optical features with micron and submicron structures, whose complexity is the main obstacle for unauthorized copying. In that sense, documents are best protected by the diffractive optical elements generated lithographically and mass-produced by embossing. The problem is that the resulting security elements are identical, facilitating mass-production of both original and counterfeited documents. Here, we prove that each butterfly wing-scale is structurally and optically unique and can be used as an inimitable optical memory tag and applied for document security. Wing-scales, exhibiting angular variability of their color, were laser-cut and bleached to imprint cryptographic information of an authorized issuer. The resulting optical memory tag is extremely durable, as verified by several century-old insect specimens still retaining their coloration. The described technique is simple, amenable to mass-production, low cost and easy to integrate within the existing security infrastructure.



KEYWORDS

biophotonics, complexity, iridescence, optical document security, variability

1 | INTRODUCTION

Insects have been used more than any other living creatures as a blueprint for design of novel devices. Butterflies and moths (order: Lepidoptera) are particularly inspiring, due to vast number of species (nearly 180 000) [1] and peculiar optical properties of their wings covered with large number (500–1000/mm^2) of tiny, overlapping scales [2] (see section 1 of Appendix S1 for a short description of their properties). Some of them are structurally colored [3] that is, produce colors by interference, diffraction and scattering, rather than pigments. This is due to complex, regular or irregular, micro/nanostructures, which can be classified in several groups according to their morphology [4]. Most frequently, iridescence (characterized by directionally dependent coloration [3]) can be observed.

Back into the XVIII century, Benjamin Franklin came up with an idea to reproduce the complexity of natural structures for document protection. He printed venation patterns of plant leaves on dollar bills to prevent counterfeiting [5]. Nowadays, his method was superseded by artificial security components, such as optically variable devices (OVDs) [6]. Diffractive optical elements (DOEs) are commonly exploited for the purpose, due to their, inherently complex microstructures, recognizable optical pattern and capability for mass-production by embossing. There is a significant drawback: for the specific type of document, all embossed copies of DOEs are identical. If a fake DOE is manufactured, counterfeited document can be made in large quantities, too. For that reason, an important goal is to invent a device which will provide unique and individual protection for each document. Protective elements should be highly complex, unique, difficult to reverse engineer and imitate. In the relevant literature, such objects are called physical one-way functions and can be realized by embedding randomly dispersed plastic, micron-sized spheres in a transparent medium and observing mesoscopic light scattering [7]. As another example, we mention using a randomized pattern of scattering from paper-based substrates [8].

Imprints of naturally occurring structures were proposed as security elements by Hamm-Dubischar [9], Biermann and Rauhe [10], and Rauhe [11], who presented the idea of document protection using biomineralized shells of radiolarians and diatoms. The protection is based on the structural complexity of their shells. The main problem is that optical effects are not particularly conspicuous, and the complexity can be assessed only at the morphological level, using scanning electron microscopy (SEM). Another problem is that structural variations among individuals of the same species seem to be small.

Whichever security element is used, it must be integrated in a security system relying on three inspection lines [6]: the first line is overt and can be visually inspected by anyone; the second is semi-covert and uses machine inspection; while the third one is covert and relies on forensic inspection with highly specialized equipment.

Here, we analyze the structural complexity, randomness, variability and uniqueness of the optical pattern of iridescent butterfly wing scales. We aim to establish their usefulness as inimitable OVDs for individualized, covert and overt, optical document security. Additionally, we investigate wing-scales as a memory medium for inscription of additional cryptographic information.

2 | STRUCTURE AND IRIDESCENCE OF *ISSORIA LATHONIA* BUTTERFLY WING-SCALES

In this section, we analyze morphological and optical features of scales belonging to the underside silver wing-patches of the Queen of Spain Fritillary, *Issoria lathonia* (Linnaeus, 1758), (see Figure 1A and section 2 of Appendix S1 for a short description of the butterfly's life history). This particular species was studied for the characteristic coloration of individual wing-scales, consisting of red, green and bluish spots randomly dispersed along a grating-like structure (see reflection microscope image in Figure 1B,C). The resulting silver color is produced by the local, additive spectral mixing [12].

Field-emission gun scanning electron microscope (FEGSEM) images reveal detailed structure of the scale's upper lamina (UL in Figure 1D). It consists of lamellar longitudinal ridges (R) regularly separated by a distance of 1.5 μm. There is, also, a fish-bone-shaped sub-wavelength grating (SW) with period of 150 nm, radiating from ridges. The interior of the scale is hollow, filled only with nano-pillars, separating UL and lower lamina (LL).

Nonlinear optical microscopy was used to analyze three-dimensional (3D) structure of wing-scales using two-photon excited fluorescence (TPEF) of chitin. Nonlinear microscope was constructed in-house [13] (see Appendix S1 for details) and used to reveal that the wing scales have irregular, wavy shape (see Figure 1E). This significantly contributes to variability of the resulting optical pattern, together with variation of the thicknesses of upper and lower laminae and their mutual distance.

We have found that the individual wing scales are iridescent, that is, the color pattern strongly depends on illumination and observation directions. The pattern has maximum brightness and sharpness for orthogonal illumination, directly through the microscope objective (resulting in an image like in Figure 1C).

3 | VARIABILITY OF OPTICAL PATTERN AND UNIQUENESS OF BUTTERFLY WING-SCALES

In this section, we will establish a connection between the wing scale morphology and the resulting reflection spectrum. To do that, we have to make a numerical model, enabling us to calculate the reflection spectrum of a single wing scale, removed from the wing and attached to a transparent substrate (as in Figure 1C). For simplicity, each scale is represented by two, wavy thin plates, separated by the layer of air. To approximate waviness each scale is divided into a number of vertical sections with different positions

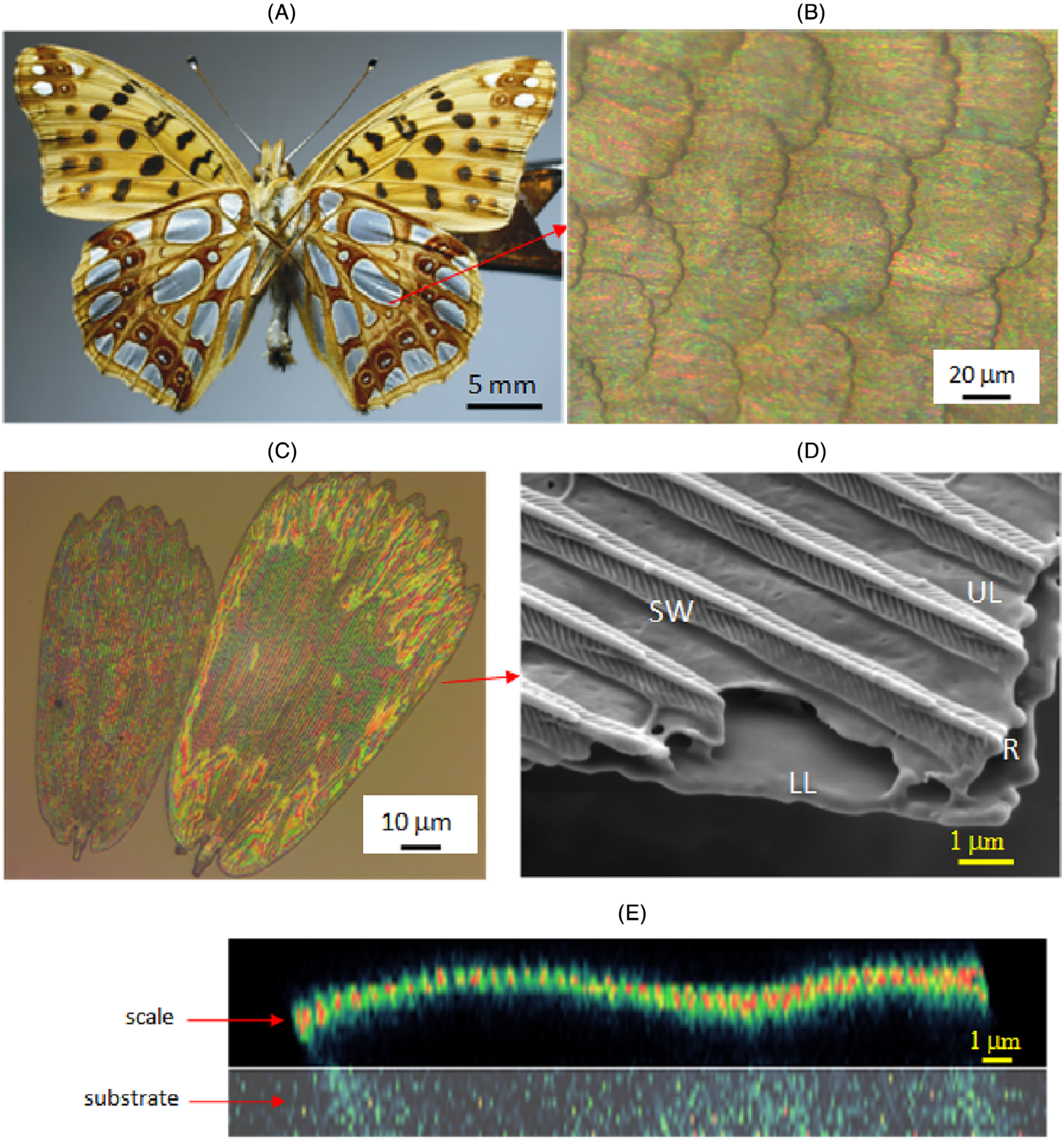


FIGURE 1 A, Ventral side of *Issoria lathonia* butterfly. B, Reflection microscope (10×, 0.25 NA) image of wing scales from the silver patch. C, Reflection microscope image (20×, 0.4 NA) image of two isolated wing-scales, removed from the wing of *I. lathonia*. D Scanning electron microscope image of the *I. lathonia* wing scale. LL and UL are lower and upper lamina, respectively, R is a ridge, while SW is a, fishbone-shaped, sub-wavelength grating. E, Wavy cross-section of butterfly wing scale (as recorded on a nonlinear optical scanning microscope)

and thicknesses of layers (Figure 2A). Each section contains two layers of chitin, the first of which was regarded as a sub-wavelength scattering surface, due to its irregularity and presence of the subwavelength grating (Figure 1D). Both layers are separated from the glass substrate by an additional air layer.

Reflection spectrum of each section was calculated using a transfer matrix method, modified to include the effects of

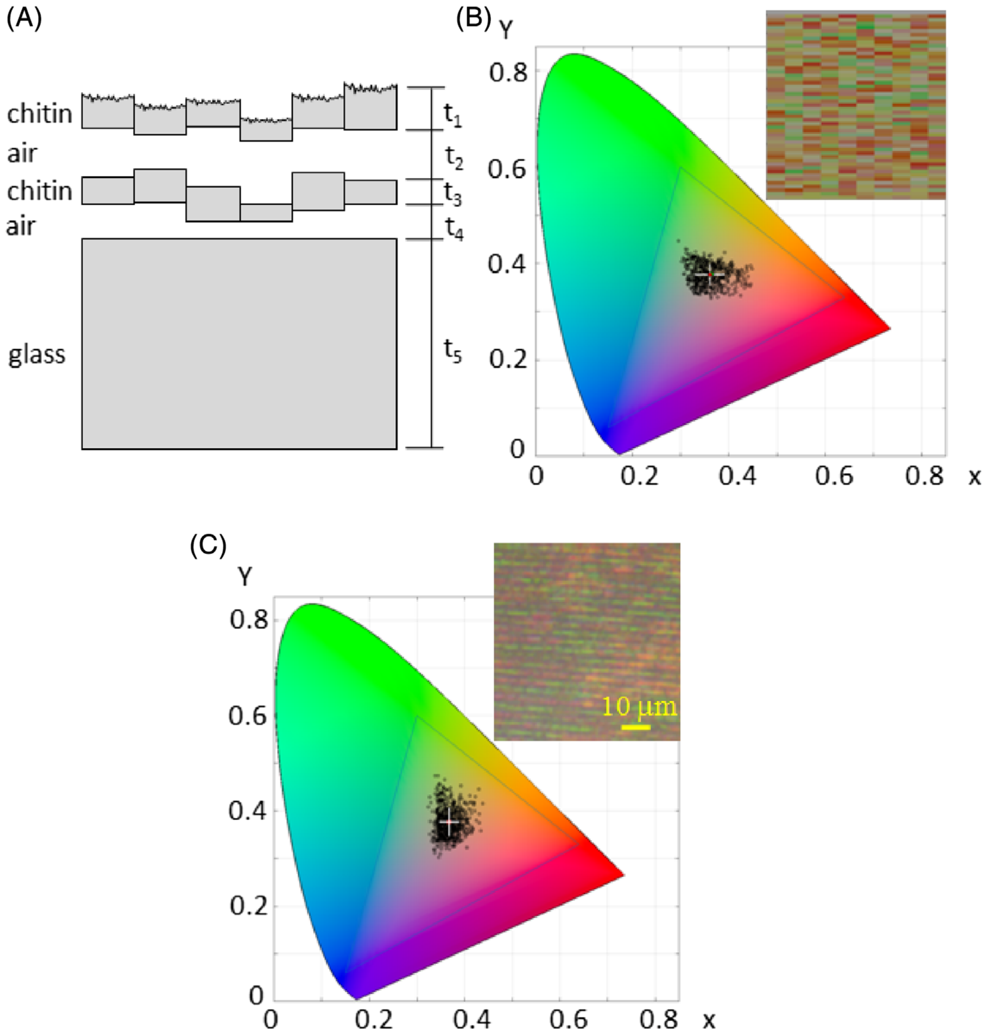


FIGURE 2 A, A theoretical model of a wing scale on the glass substrate. B, Color coordinates of a modeled pattern are presented in a CIE 1931 diagram, together with the color pattern in the inset. C, Color coordinates of *Issoria lathonia* pattern are presented in a CIE 1931 diagram. A section of a *I. lathonia* wing scale pattern, used to calculate color coordinates, is presented in the inset. Crosses in B and C represent average color value and their lengths indicate SDs in *x*- and *y*-directions

scattering [14]. Layer thicknesses (t_1, t_2, …, t_5 in Figure 2A) and their corresponding refractive indices were the parameters of the model, as well as the root mean square roughness (RMS) of the surfaces.

To simulate the wing scale as a whole, the same calculation was performed for each section. The starting point of our simulation was the layer thicknesses estimated from Figures 1D,E) (~100 nm chitin, ~1000 nm air layer thickness—see section 3 of Appendix S1 for the complete list of parameter values). Layer thicknesses were stochastically varied (according to normal distribution) with pre-defined SD σ = 15 nm. Following the calculation of spectrum for each section, *xyY* color coordinates were calculated. They were presented in a CIE 1931 diagram (black dots in Figure 2B), which was designed to closely match human color perception (through three color-matching functions) [15]. It is, also, a useful tool to represent RGB values of color-camera images recorded through this research.

Calculated colors are, also, represented as a pattern of rectangular colored patches (see inset in Figure 2B). For comparison, color coordinates of experimentally recorded pattern (inset in Figure 2C) were also computed and presented in CIE 1931 diagram (Figure 2C).

We were not able to obtain perfect match in CIE diagrams (Figures 2B,C), for the same reason which prevents a counterfeiter to forge a wing scale—complexity of the problem. However, we were able to match the position of the mean color coordinate (small white crosses in CIE diagrams) of theoretical and experimental image. The shape of the color scattering distribution is different, but the SDs are similar.

The most important result is that the variation of layer thicknesses by only ±15 nm leads to experimentally recorded variability of coloration. This means that one trying to copy the exact coloration pattern of the wing scale, has to maintain an extreme precision of manufacturing—at least one-tenth of the layer thickness variability (~1.5 nm). The task is well beyond practical limits of modern technology, and cellular noise precludes replication of identical wing-scales by natural means.

Wing-scales described above have a sufficient number of degrees of freedom (in terms of layer thicknesses and waviness) to enable significant variability. Here, we want to find how difficult would be to find two identical scales.

We first analyze the statistical properties of the wing-scales color patterns by decomposing an image into its RGB components and calculating two-dimensional (2D) autocorrelation function for each color channel separately—see details in section 4 of Appendix S1. It can be seen (Figure S1) that autocorrelation peak is asymmetrical, that is, its width along the wing-scale grating was estimated at 30 μm, while in the orthogonal direction it is 1.5 μm.

By taking into account that average dimensions of the scales are 50 × 100 μm, we can easily calculate that there are $[50/1.5] \times [100/30] = 33 \times 3 = 99$ (numbers were rounded to the nearest integer) statistically independent, colored patches. We can discriminate intensity of a single color channel in, at least, 10 discrete levels—easily achievable for any low-cost or mobile phone camera. In that case, we may estimate that there are, at least theoretically, 10^{99} wing-scales with different patterns per every channel. Thus, finding a scale exactly the same as another, previously chosen, one is impossible from any practical point of view.

Each wing-scale is a dead remnant of an individual cell and thus reflects intrinsic randomness of cellular development. This is a natural consequence of cellular noise [16], which is a well-established fact in biology, resulting in non-deterministic relation between genotype and phenotype. The important thing about butterfly wing scales is that they "freeze" the cellular noise, by leaving it in a state just before the cell died. Cellular noise cannot be switched-off and it is expected to be similar in all other butterfly species. In that sense, the similar level of randomness is expected on all wing-scales of all butterflies [17] including those of the *Issoria lathonia* species.

4 | OPTICAL DOCUMENT PROTECTION WITH WING SCALES

The main idea of this research is to use butterfly wing-scales as a natural, hologram-like, OVDs, permanently attached to a document (eg, a plastic credit card). In contrast to artificial OVDs, natural ones are unique (guaranteed by the cellular noise) and difficult to copy (due to their layered, micro- and nano-scale patterns).

We decided to use a near-field color pattern as a security feature of a document protection system and read it under the optical microscope. Practical inability to place a document at exactly the same position and orientation within the reading system requires shift- and rotation-invariant pattern recognition algorithm. We decided to use algorithm based on Fourier-Mellin transform (FMT) [18] which fulfills the above requirements.

Nine *I. lathonia* wing-scales were attached to a glass substrate and their reflection microscope images were recorded at several positions and orientations (55 images in all). The recorded images were first decomposed into RGB components and the green one (G) was transformed using FMT. Correlations between corresponding FMT pairs were calculated and the corresponding statistical distribution is shown in Figure 3. The correlation coefficient, corresponding to the same wing-scale at displaced positions, had typical values around 0.4, while it never had values below 0.1. The most frequent values of correlation coefficient for two different wing-scales were around 0.02, and were never larger than 0.06. By placing validity threshold at 0.08, correct discrimination between wing scales is guaranteed.

To correct for accidental tilt or defocusing of the wing scale image, we have recorded images at 3 to 4, closely spaced, focal positions. Consequently, focus stacking algorithm was used (using Picolay free software) to extract well-focused parts in each recorded image and combine them in a single, sharp image.

In order to build a strong security system, malicious party has to be prevented from picking any butterfly wing-scale and attaching it to a document. This can be performed by making a document self-verifying by using a digital signature of the document issuer, within the public key infrastructure (PKI) system [19]. Here, we show that the necessary authentication information can be written on the wing-scale itself.

We used femtosecond laser-processing to additionally modify butterfly wing scales and exploit them as a write-

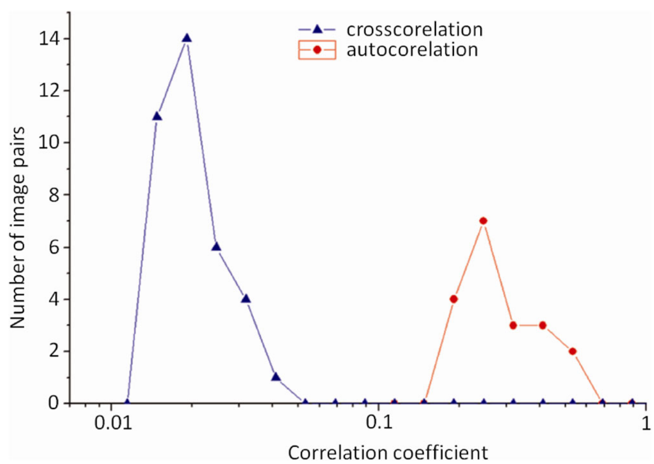


FIGURE 3 Cross- and auto-correlations of ensemble of 55 pairs of wing-scale images. Graph shows a number of image pairs vs the corresponding correlation coefficient. Maximum cross-correlation coefficient is at 0.02, while autocorrelation coefficient is always above 0.2

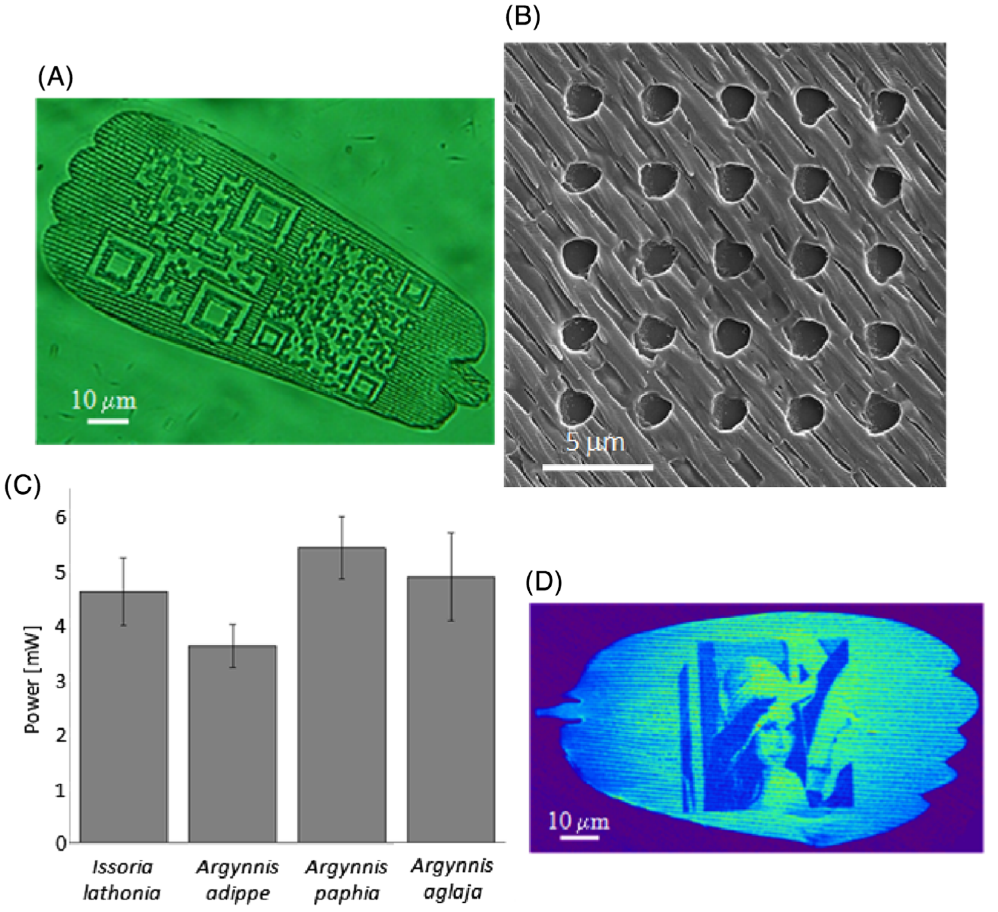


FIGURE 4 (A) Transmission microscope image of a femtosecond-laser-cut wing scale (QR-codes). (B) Array of holes on a *Issoria lathonia* wing-scale showing the minimum achievable diameter of a laser cut. (C) Thresholds for laser cutting of four butterfly species used throughout this research. (D) Selectively bleached wing scale with a Lena image observed by fluorescence modality of a nonlinear microscope

only memory. The software of a home-made nonlinear-microscope [13] was modified to enable vector and raster drawing of an arbitrary image (see section 5 of Appendix S1 for additional details). Depending on the average laser power, repetition rate and dwell time, wing scale can be cut (as in Figure 4A)). Minimal diameter of a laser cut achieved throughout this research is 1.7 μm, as shown in Figure 4B. Damage threshold is 4.5 mW (using 40 × 1.3 NA microscope objective) and 8.0 mW (with 20 × 0.8 NA microscope objective). Three more butterfly species were analyzed in that respect, with similar damage thresholds (Figure 4C). In practice, we operated above threshold to enable reliable and repeatable laser-drawing. That is why we achieved the minimum cut width which is considerably above the lateral resolution of our femtosecond system [13]. Based on that and the average size of the wing-scale (~50 × 100 μm^2), we estimated the information capacity of a single scale at about 3000 bits, providing that the damaged spot is treated as binary 1, and undamaged as binary 0.

Here, we point out that each bit, written on the wing-scale, reduces the number of statistically independent patches. We will assume that one half of the wing-scale surface is laser processed (reducing the original wing-scale area of 50 × 100 = 5000 μm^2 to approximately 35 × 70 = 2450 μm^2). That leaves approximately [35/1.5] × [70/30] = 23 × 2 = 46 colored patches (numbers are, again, rounded to the nearest integer). Thus, as in the previous section, we may estimate the number of different wing scales at 10^{46} (per every RGB channel), each one being protected by 1500 bits of additional information.

By reducing the laser power below the damage threshold, we were able to bleach the autofluorescence of the wing-scale and use it to inscribe covert information (Figures 4D) as a gray level image.

5 | DISCUSSION AND CONCLUSIONS

While speaking of document protection, an important question immediately comes to mind: how difficult it is to counterfeit wing-scale? Forgeries can be produced by either (a) imitating the structure or (b) imitating the corresponding optical effect with another, possibly simpler, structure. The first approach is based on "reverse-engineering" and manufacturing of identical protective element structure, while the second one is based on imitating the optical effect.

Reverse engineering of butterfly wing-scales implies analysis of the 3D morphology and material properties (refractive index and absorption) followed by some-kind of lithographic copying of both the morphology and material properties. Even with the most advanced technologies (microtomography, electron or X-ray holography), this approach will be extremely limited in terms of available resolution of 3D analytic and lithographic methods (of the order of 10 nm), duration and cost [20].

Imitating the optical effect requires careful analysis of iridescence across the whole visible spectrum and angular range, followed by finding a method to faithfully reproduce the optical wavefront. This also poses a fundamental question: is it possible to have identical wave-fields generated by different structures? The question goes into scattering theory, with a plethora of papers dealing with the uniqueness of the direct and inverse problems. There is no general answer to the question, because it depends on the nature of the scatterer (penetrable or non-penetrable), boundary conditions (conductive, dielectric, amplifying), wavelength and angular range of probing radiation [21]. There are more or less exotic situations where uniqueness is not guaranteed, such as amplifying medium or medium with optical cloaks [22]. But, for the range of problems relevant to this work, the answer is no—there are no two different scatterers producing the same scattered field (far or near) [23].

The wing-scales are best protected by their uniqueness implying necessity to counterfeit every single document time and again. Another point is that, both the material composition and morphology are unique, producing a plethora of optical effects: overall shape, iridescence, absorption, polarization, fluorescence, moiré, defects, far and near-field diffraction pattern, local spectra, etc. In addition, scales possess different optical properties on their upper and under side, which may be used to produce security features which can be read from both sides in perfect alignment (so-called see-through register). Simultaneous use of all or some of the mentioned effects vastly increases the capabilities of wing scale as a protective element.

An important question is whether wing scales can be copied by some of holographic methods. Up to now, volume and surface relief holograms have been copied using contact [24], non-contact [25] or scanning [26] methods. However, these techniques are not useful for copying step-index, layered structure of wing scales, because of the sinusoidal nature of holographic gratings. Additionally, subwavelength gratings of wing-scales (S in Figure 3) cannot be copied, due to evanescent fields obtained by diffraction. These tiny structures are essential for the final coloration of the wing scale, because they produce uniformly scattered radiation in the blue part of the spectrum (see blue component of the wing scale pattern in Figure 8A).

It should be emphasized that Lepidoptera species are not equally suitable for document protection. As already mentioned, these structures must have complex nanometer to micron-size features, with significant variability and must be difficult to analyze and reverse engineer. We preferred nymphalid species, possessing silver patches on their wings. Other Lepidoptera species, with structurally colored scales have been tested. However, the scales of these species were not so easy to process and manipulate, with the equipment at our disposal.

There is a number of ways how insect scales can be manipulated and attached to documents, as described in the following patent applications [27–29]. Generally speaking, they have to be, either embedded within the transparent medium with large refractive index difference (compared to that of the scale), or placed in a recess with a transparent, protective, covering. The procedure can be performed by micromanipulation or by standard printing techniques (silk-screen, flexo-printing).

Once embedded, scale contents have to be read by some means, which depends on the insect species, type of the scales and the optical effect sought for. In addition to iridescence pattern detection described above, there are other choices: overall shape of the scale, near field color pattern, far-field diffraction pattern, moiré pattern, or pattern of defects (looking like minutia in a fingerprint), with many variations (such as phase and amplitude) and combinations (by recording simultaneously several effects). Reading devices can be based on far- or near-field detection, holography or scanning techniques using CD/DVD readout heads. In the context of document protection, strong variability of patterns with angular position of illumination and observation, as well as the polarization sensitivity are very important. This is what prevents malicious attacks by simple color laser-printing.

The document protection described here is limited to machine reading level. It can be extended to the forensic level, by reading electron microscope image (Figure 1D)), with, for example, cross-rib distances serving as a random feature. If visual protection is desired, a large number of scales can be transferred to another substrate, so to cover large area, visible with the naked eye. One of the scales can be chosen for machine and forensic protection, as described in Reference [28].

Practical implications of the proposed document protection method are numerous. There are thousands of wing scales on a single butterfly specimen suitable for document protection (we have estimated 40 000 iridescent wing scales on *I. lathonia* silver spots). With appropriate choice of butterfly species (eg, Morpho spp.) this number can be much larger. If commercially available dry butterfly specimens are used, we have estimated the cost of a single wing scale at $85{\cdot}10^{-6}$ \$. Alternatively, butterfly species can be reared using well-established techniques of sericulture (silkworm raising). Wing-scales can be collected cheaply and applied using any of standard printing techniques (silk-screen, offset, ink-jet). Range of applications is huge: banknotes, creditcards, CD/DVDs, bonds, valuable goods. It is not even hard to imagine using wing-scales as a hardware lock for digital information security.

The base material of wing-scales is chitin, which is extremely and verifiably durable. Natural history museums have century-old butterfly specimens retaining their structural coloration and we have more than 30 years old specimens of *I. lathonia* with silver patches as shiny as in live insects. Even more, fossilized insects retain their iridescence after petrifaction and last for millions of years [30]. This should be compared to, recently described, five-dimensional optical memory [31], claiming "seemingly unlimited lifetime."

Wing scales may reversibly change their dimensions in response to temperature variation [32], humidity and vapors [33]. As a consequence, there is a slight spectral shift, but it is too small to affect application of wing scales in document security, under normal atmospheric conditions. Systematic changes during extended periods of time are not expected due to hydrophobicity, insolubility and biological inertness of wing-scales [34]. However, we have not measured the long-term stability of wing scale patterns, we plan to perform accelerated aging tests in the near future and reveal details regarding the effect of aging on pattern stability.

Anyway, the validity period of most documents is less than 10 years, a period during which wing scales are expected to remain unaffected. Furthermore, taking into account the chemical and physical stability of chitin and the fact that optical response of the insects a hundred and more centuries-old (from museum) and from fossil samples exhibit extraordinary similarity with visual response measured from "the fresh" samples, suggest that corresponding patterns are very stable and could have long-term cryptographic applications.

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CONFLICT OF INTEREST

Institute of Physics Belgrade is the applicant and the owner of three pending patent applications given in a list of references [27–29] whose contents is partially described in this paper. Five authors (D.V.P., D.P., M.D.R., V.L. and A.J.K.) are also authors of abovementioned patent applications. Specific aspects of manuscript covered in patent applications are: laser cutting and bleaching of wing scales, as well as a partial list of butterfly species usable for this purpose.

AUTHOR CONTRIBUTIONS

D.V.P. conceived the idea; M.D.R., A.J.K. and D.V.P. have constructed the nonlinear microscope used in this research. D.P., A.J.K., M.D.R. and V.L. performed experiments and measurements. D.V.P. designed an optical model of wing-scales, while N.V. and D.S. performed the theoretical analysis of uniqueness of butterfly wing-scales. B.J. D. Z., W. Z. and B. K were included in data analysis and supervised the research. D.S., S.Ć and D.P. made adequate choice of appropriate butterfly species used in this research, while D.V.P., D.P. and M.D.R. prepared the manuscript, based on comments of other authors. This work is performed in partial fulfillment of the requirements for the PhD degree of Vladimir Lazović at the University of Belgrade, Faculty of Physics. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

ETHICAL STANDARDS

Insects used in this research are NOT on the list of strictly protected and protected species defined in: By-law on proclamation and protection of strictly protected and protected wild species of plants, animals and fungi, Official gazette of the Republic of Serbia Nos. 5/2010 and 47/2011. All experiments were performed on dry specimens from the collection of Dejan Stojanović. The research did not include live insects.

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REFERENCES

[1] G. F. Robert, P. H. Adler, *Insect Biodiversity, Science and Society*, Chichester, UK: Willey-Blackwell, **2009**.

[2] X. Zhou, S. W. Applebaum, M. Coll, *Environ. Entomol.* **2000**, *29*, 1289.
[3] S. M. Doucet, M. G. Meadows, *J. R. Soc. Interface* **2009**, *6*, S115.
[4] W. Zhang, J. Gu, Q. Liu, H. Su, T. Fan, D. Zhang, *Phys. Chem. Chem. Phys.* **2014**, *16*, 19767.
[5] E. P. Newman, *Proc. Am. Philos. Soc.* **1971**, *115*, 341.
[6] R. L. Van Renesse, *Optical document security*, 3rd ed. Norwood, MA: Artech House, **2004**.
[7] R. Pappu, B. T. J. Recht, N. Gershenfeld, *Science* **2002**, *297*, 2026.
[8] J. D. R. Buchanan et al., *Science* **2005**, *436*, 475.
[9] Hamm-Dubischar, C. Patent No. WO 2007031077 (A1), Inorganic marking particles for characterizing products for proof of authenticity method for production and use thereof (**2007**).
[10] Biermann, N. & Rauhe, H., Method for Producing Security Markings. *WO2004070667 A2* (**2004**).
[11] Rauhe, H., Verfahren zur Herstellung von informationstragenden mikropartikulären Gemischen. Patent DE10238506 A1 (**2004**).
[12] P. Vukusic, R. Kelly, I. Hooper, *J. Roy. Soc. Interface* **2009**, *6*, S193.
[13] D. M. Rabasović et al., *J. Biomed. Opt.* **2015**, *20*(016010), 1.
[14] D. Pantelić, S. Savić-Šević, D. V. Stojanović, S. Ćurčić, A. J. Krmpot, M. Rabasović, D. Pavlović, V. Lazović, V. Milošević, *Phys. Rev. E* **2017**, *95*, 032405.
[15] J. e. Schanda, *Colorimetry: Understanding the CIE System*. Hoboken, NJ: John Wiley & Sons, **2007**.
[16] I. G. Johnston et al., *PLoS Comput. Biol.* **2012**, *8*, e1002416:1.
[17] A. Dinwiddie, R. Null, M. Pizzano, L. Chuong, A. Leigh Krup, H. Ee Tan, N. H. Patel, *Dev. Biol.* **2014**, *392*, 404.
[18] J. Wood, *Pattern Recog.* **1996**, *29*, 1.
[19] L. O'Gorman, I. Rabinovich, *IEEE Trans. Pattern Anal. Mach. Intell.* **1998**, *20*, 1097.
[20] K. Watanabe, T. Hoshino, K. Kanda, Y. Haruyama, T. Kaito, S. Matsui, *J. Vac. Sci. Technol. B* **2005**, *23*, 570.
[21] D. Colton, R. Kress, *Inverse Acoustic and Electromagnetic Scattering Theory*, 2nd ed. New York, NY: Springer, **1998**.
[22] F. Monticone, A. Alu, *Phys. Rev. X* **2013**, *3*(041005), 1.
[23] G. Bao, P. Li, J. Lin, F. Triki, *Inverse Probl* **2015**, *31*, 093001.
[24] S. Toxqui-López, A. Olivares-Pérez, I. Fuentes-Tapia, A. Quintero-Romo, *Opt. Mater.* **2007**, *29*, 1604.
[25] B. D. Chrysler, R. K. Kostuk, *Appl. Optics* **2018**, *57*, 8887.
[26] M. Okui, K. Wakunami, R. Oi, Y. Ichihashi, B. J. Jackin, K. Yamamoto, *Appl. Optics* **2018**, *57*, 4117.
[27] Pantelic, D., Rabasovic, M., Krmpot, A., Lazovic, V. & Pavlovic, D., Security device individualized with biological particles. PCT/EP2015/081398 (**2015**).
[28] Pantelic, D., Rabasovic, M., Krmpot, A., Lazovic, V. & Pavlovic, D., Security tag containing a pattern of biological particles. PCT/EPO2015/081400 (**2015**).
[29] Pantelic, D., Rabasovic, M., Krmpot, A., Lazovic, V. & Pavlovic, D., Security tag with laser-cut particles of biological origin. PCT/EP2015/081407 (**2015**).
[30] M. E. McNamara et al., *PLoS Biol.* **2011**, *9*, e1001200:1.
[31] J. Zhang, M. Gecevičius, M. Beresna, P. G. Kazansky, *Phys. Rev. Lett.* **2014**, *112*, 033901.
[32] A. D. Pris, Y. Utturkar, C. Surman, W. G. Morris, A. Vert, S. Zalyubovskiy, T. Deng, H. T. Ghiradella, R. A. Potyrailo, *Nat Photon* **2012**, *6*, 195.
[33] R. A. Potyrailo, H. Ghiradella, A. Vertiatchikh, K. Dovidenko, J. R. Cournoyer, E. Olson, *Nat photonics* **2007**, *1*, 123.
[34] H.-M. Hu, J. A. Watson, B. W. Cribb, G. S. Watson, *Biofouling* **2011**, *27*, 1125.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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Naturally safe: cellular noise for document security

Supplemental Document – Materials and methods

1. Properties of butterfly scales

A standard, non-specialized scale, consists of two parallel plates (laminae), joined with pillar-like structures - trabeculae. The lower lamina, facing the wing membrane, is smooth and featureless, while the upper lamina, facing outward, is intricate, with a complex architecture of longitudinal ridges connected at intervals by cross-ribs. These form a series of “windows” to the hollow interior of the scale. The ridges are covered with two systems of folds: longitudinal (overlapping lamellae), and microribs (perpendicular to lamellae), covering the sides of the ridges.The remaining groups are elaborations of the standard scale, and the classification is based on which region of the scale is modified. Modifications include a wide diversity of specializations of the ridges, cross ribs, as well as formation of body-lamine and 3D photonic crystal structures.

2. Insects used in this research

The Queen of Spain fritillary (*Issoria lathonia*, (Linnaeus, 1758) – see Fig. S1) is a butterfly belonging to Nymphalidae (Insecta: Lepidoptera) family. The species is widespread from West Europe, across North Africa and Central Asia to the Himalayas and West China. It inhabits sunny, opened meadow habitats at altitudes between sea level and 2700 m. *I. lathonia* appears in three annual generations and flies from March to the end of October. The larvae feed on plants from *Viola spp* [1]. According to the IUCN categorization, species is not threatened in Europe [2].

Not all Lepidoptera wing scales are equally suitable for document protection. There are several criteria which must be fulfilled in order to use the scales as a security device. As already mentioned, these structures must have complex nanometer to micron-size features, with significant variability and must be difficult to analyze and reverse engineer. We preferred Lepidoptera species which possess silver patches on their wings. Four Palearctic nymphalid species were used in this study: *I. lathonia*, *Argynnis adippe* (Denis & Schiffermüller, 1775), *Argynnis paphia* (Linnaeus, 1758), *Argynnis aglaja* (Linnaeus, 1758) (Lepidoptera: Nymphalidae). There are several other Lepidoptera species, with structurally colored scales, that have been tested, such as: *Diachrysia chrysitis* (Linnaeus, 1758), *Autographa gamma* (Linnaeus, 1758), *Autographa jota* (Linnaeus, 1758), *Jordanita globulariae* (Hübner, 1793), *Callophrys rubi* (Linnaeus, 1758), *Apatura ilia* (Dennis & Schiffermueller, 1775) and *Apatura iris* (Linnaeus, 1758). However, the scales of these species were not so easy to process and manipulate, with the equipment at our disposal.

Preserved, dry specimens of the four butterfly species, used through this research were borrowed from the private collection of Dejan Stojanović (Novi Sad, Serbia). A number of specimens were collected on Mt. Fruška Gora, Serbia, during the flight period, in 2013. Several specimens of *Issoria lathonia* were collected 34 years ago (by Dejan Stojanović).

3. Parameters of a model of butterfly wing-scales

To simulate experimentally recorded wing scale pattern from Fig. 4(a), we used the following parameters:

Refractive index of material	1.57
Refractive index of air	1
Average thickness of material	108 nm
Average thickness of air layer	969 nm
RMS roughness of the layer	59 nm
Variability of layer thickness	±15 nm
Angle of incidence variability	±3 deg

It is true that the inverse scattering problem is ill-posed [3], opening the possibility of having two different scatterers producing marginally different scattering patterns. However, the scatterers will be widely different, even generating unphysical solutions. To make them physical, regularization (using some *a priori* information – such as the shape of the wing scale) is necessary, requiring production of a structure almost identical to the original one.

4. Autocorrelation properties of a wing scale pattern

A wing scale image, such as one in Fig. S1(a) was used to analyze statistical properties of a pattern. We wanted to perform analysis without taking into account the overall shape of the scale. That is why we used a cropped rectangular part of the scale and only one of its three color channels (Fig. S1(b)). 2D autocorrelation function (Fig. S1(c)) was calculated using Gwyddion 2.52 free visualisation and analysis software tool, and the corresponding image is shown in Fig. S1(b).

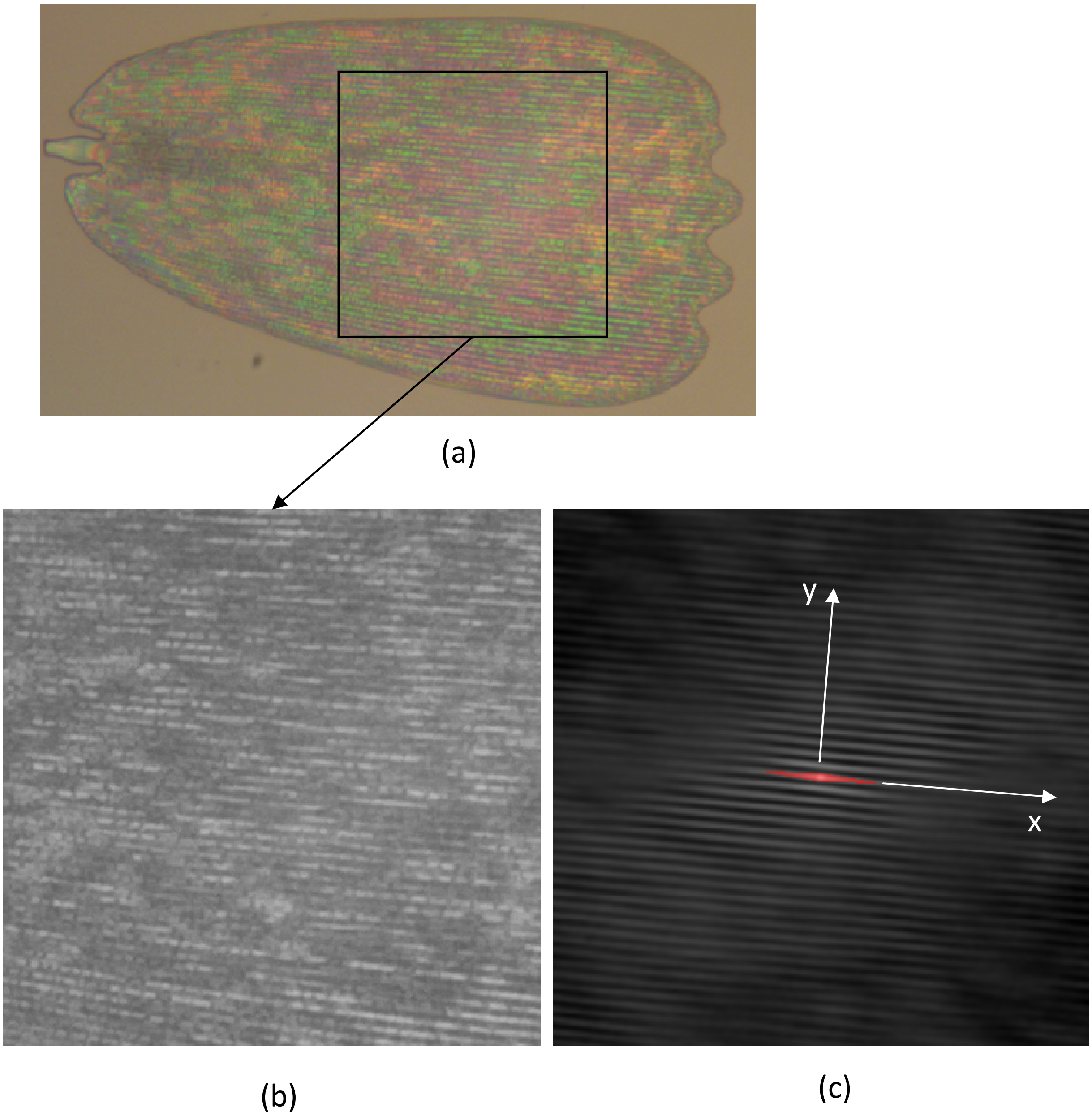


Fig S1: (a) Reflection image of a wing scale, whose part (outlined with the black square) was cropped and its (b) green color component used in a further calculation of (c) 2D autocorrelation function. Red area in the center designates region above the autocorrelation level of 0.2.

Autocorrelation function is oscillating along y-axis and decaying more slowly along x-axis. We assumed that the autocorrelation below 0.2 is small enough for two pattern sections to be statistically independent. That area is designated with red, and its length along x-axis is approximately 30 μm, while along y-axis is 1.5 μm. By taking into account that the average size of the wing scale is of the order 50 x 100 μm, we can estimate that there are approximately [50/1.5] × [100/30] = 33 × 3 = 99 statistically independent elements of a pattern.

5. Femtosecond microscopy and laser-cutting of wing scales

Through this research, we used home-made nonlinear microscope, described in detail in [4]. In short, a beam from a femto-second laser (Mira, Coherent) was scanned through a modified Zeiss microscope, enabling excitation of two-photon auto-fluorescence of chitin. We mostly used Carl Zeiss objectives: Plan–APOCHROMAT 20x, NA 0.8 and EC Plan-NEOFLUAR 40x, NA 1.3 oil immersion. Images were acquired using laser power less than a mW with the best lateral and axial resolution of 0.7 μm and 2.1 μm, respectively. Bright-field images were captured on a Canon EOS 50D digital camera.

To reveal the cross-sectional geometry and further contribute to the individualization of the scales, we cut them with a femto-second laser beam using a microscope described above. Different patterns and lines were made on the surface of individual scales using a nonlinear microscope setup. Information was inscribed by cutting through scales or by bleaching their fluorescence. The laser wavelength, scanning speed and power are chosen to enable cutting, engraving or bleaching. Beam power was increased above the threshold level and software was modified to enable drawing arbitrary shapes using vector images.

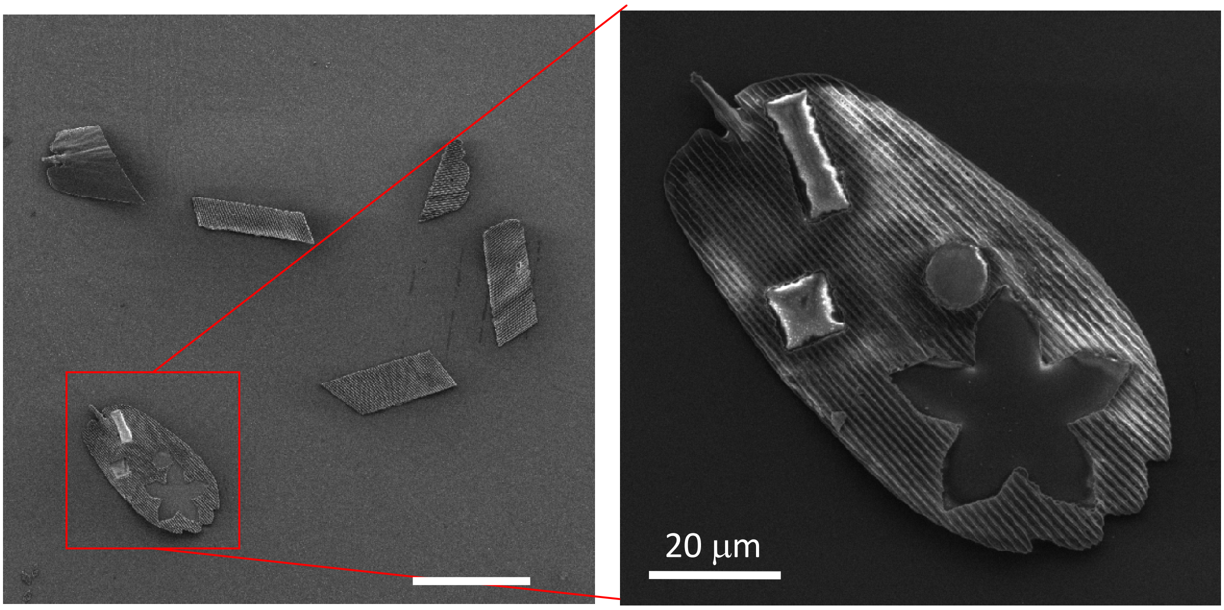
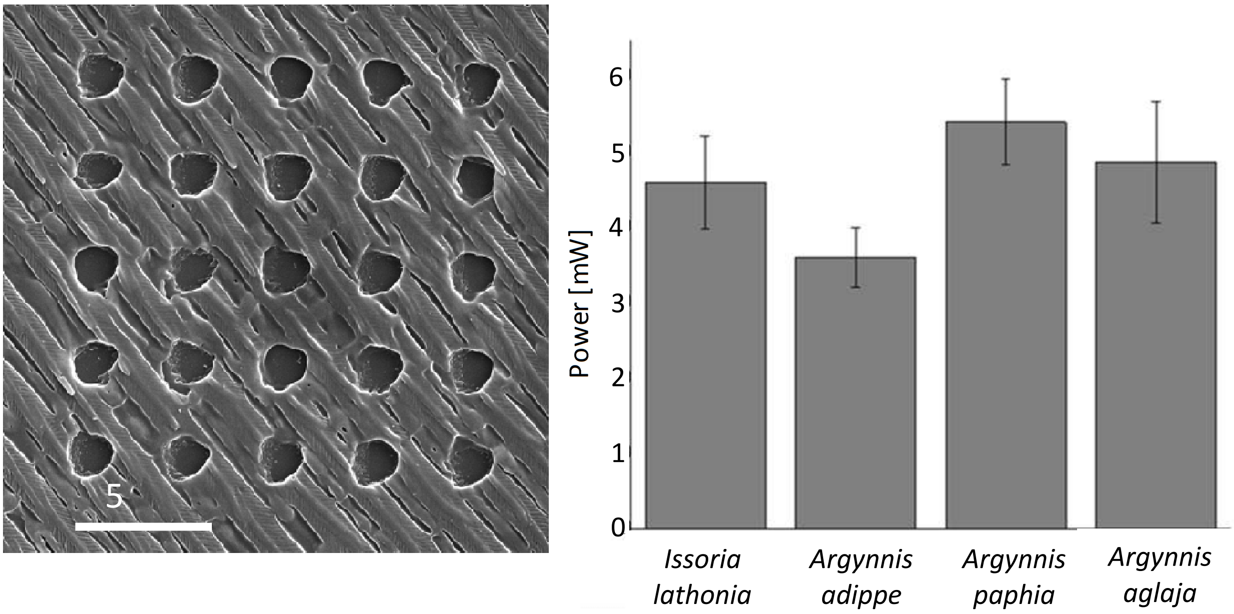


Fig S2: SEM images of femtosecond-laser-cut wing scales

Individual scales were manipulated under the microscope, detached from the butterfly wing and transferred to the microscope cover glass. Another cover glass was used to sandwich the scale, making it possible to treat front- and under-side. There was the problem of scale deformation, which can be either natural or induced during the transfer and positioning. This was solved by inducing water condensation between the substrate and the scale, followed by straightening of the scale due to surface tension. After evaporation of the water, scale remained straight and attached to the substrate.

Wing scales were first imaged by detecting autofluorescence signal obtained upon two-photon excitation. Instrument is then placed into vector drawing mode and a desired pattern is loaded into the computer. Femto-second laser power was increased above the threshold and the wing scale was cut (see SEM image S2). To find the damage threshold we have been drawing simple patterns (such as one in Fig. S3 (a)) with different laser power. We have found that the threshold depends on the butterfly species (*Issoria lathonia*, *Argynnis adippe*, *Argynnis paphia*, *Argynis aglaja* were used), wavelength and the microscope objective used. The lowest thresholds were obtained using 730 nm wavelength and 40x, 1.3NA microscope objective (see Fig. S3(b)). It is important to say, that cutting was possible only in femto-second regime of the laser. When placed into CW mode cutting was impossible, even with the highest power supplied by our laser. This proves that the multiphoton processes are responsible for laser cutting.



(a) (b)

Fig S3: (a) A pattern used to test damage threshold and resolution of laser writing. (b)Wing-scale damage threshold of four butterfly species cut using immersion microscope objective 40x, NA1.3 at 730 nm wavelength.

6. Microscopic analysis

Optical characteristics of butterfly wings and wing-scales were observed using a transmission and reflection microscope. Observations were made on the entire silver patch, directly on the wing, and on the individual scales removed from the wing. Wing scales were manually detached from the butterly's wing and attached to the glass substrate. They possess natural waviness which required focus stacking to obtain sharp microscopic images. To eliminate the focusing problem, we used condensation of water vapor to flatten the scale by surface tension. After evaporation, scales remained flat and firmly attached to the substrate by adhesive forces. Analysis was done on, both, cover and ground scales, although there are no significant differences in the structural pattern among them. They only differ in size – the cover scales being larger than the ground ones. Detailed, submicron structure of the scales, were studied using a field-emission gun scanning electron microscope (Mira, Tescan).

References

1. Tolman, T. & Lewington, R., *Butterflies of Britain and Europe (Collins Field Guide), 2nd edition* (Collins, 2004).

2. Van Swaay, C. *et al.*, *European red list of butterflies* (Publications Office of the European Union, Luxembourg, 2010).

3. Bao, G., Li, P., Lin, J. & Triki, F., Inverse scattering problems with multi-frequencies. *Inverse Problems* **31**, 093001 (2015).

4. Rabasović, D. M. *et al.*, Nonlinear microscopy of chitin and chitinous structures: a case study of two cave-dwelling insects. *J. Biomed. Opt.* **20**, 016010:1-10 (2015).