



Reduced ASE threshold from aperiodic photonic structures in rhodamine B-doped king salmon (*oncorhynchus tshawytscha*) iridophores

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ABSTRACT

The broadband reflectance of iridescent silvery fish is caused by aperiodic photonic structures formed by complex distributions of guanine platelets in their iridophores. Photons can be reflected multiple times inside the iridophores found in many pelagic teleosts allowing for advanced photonic methods of characterization. The one-dimensional aperiodic structures reflect light over a broad spectrum. Enhanced amplified spontaneous emission (ASE) and suppressed laser light in stained skin tissue is attributed to the high group velocities and negligible localization of light over the gain envelope of rhodamine B (RhB). The enhancement to the ASE, reported herein, is shown to be caused by an increase in the effective ASE gain path length of photons generated in the iridophores of skin tissue stained with RhB. We show that the effective ASE gain path length can be used as a figure-of-merit to quantify aperiodic photonic structures, including those found in many underwater animals, by determining the increase of a photon's average time spent in broadband reflective tissue.

1. Introduction

An iridophore is a specific type of animal chromatophore cell (color cell) which does not derive its color from dyes or pigments. Rather, an iridophore contains complex structures that reflect light at multiple interfaces. Some fish iridophores are highly reflective with low dispersion over the visible spectrum while other iridophores have well-defined photonic crystal structures with distinct reflection bands caused by the constructive and destructive interference of light at different wavelengths [1–4]. The structural color of reflected light from iridophores found in fish species depends on the size, shape, orientation, and distribution of guanine platelets [5,6]. Due to the high parameterization of photonic crystal structures in fish iridophores and the heterogeneous distribution of chromatophores in general, the amplitude, color, and polarization of the reflected light largely depends on an observer's orientation with respect to a fish and the light source [7,8]. Many fish use their iridophores as camouflage [9], while other species of fish have developed photonic crystal structures for communication [10]. The broadband reflections observed in many open-ocean teleosts is caused by uneven stacks of guanine platelets, such as those with chaotically distributed or chirped thicknesses/spacings [11,12]. Various types of guanine crystal platelets are also present in the broadly reflecting

iridophores of pelagic teleosts [11,12].

Spatial- and temporal-coherent photon emission has been observed in cells using both external cavities [13–17] and intracellular resonances [18,19]. Lasing in biological tissues composed of multiple cells and/or the extracellular matrix have also been reported [20,21], where the biological material is either used as the gain medium [22], the resonant structure/cavity [23–26], or both [27]. Prior to lasing in biological materials most studies on iridophore networks involve reflection and scattering measurements of an external light source. To date, several optical techniques have been developed to quantify the structural color of animals [28–33].

Laser light emission, including emission from any biological structures, is sensitive to structural/cavity resonances [34–36]. In some circumstances, it may be advantageous to decouple the gain from any passive cavity that can result in lasing. A population inversion without resonant feedback can lead to amplified spontaneous emission (ASE) in the gain medium [37], where some recent studies have focused on the ASE from organic dyes in tissues and other biomaterials [38–46]. In this paper, a sensitive figure-of-merit for broadband reflecting iridophores is introduced, which is based on the path length of photons traveling through the gain region of fish skin stained with a laser dye. The ASE threshold condition couples the population inversion density with the

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path length of emitted photons. The effective gain path length is defined as the average path length of an emitted photon through a material before it is either absorbed or exits the gain medium. ASE has lower temporal coherence relative to laser light emitted from the same gain medium. The generation of highly coherent laser light requires feedback from dispersive cavities or other passive structures, whereas the coherence of ASE light is decoupled from the resonances of passive structures. Samples of fish skin sectioned from the head of a king salmon (*Oncorhynchus tshawytscha*) [47] fish with a high density of iridophores are used to illustrate how the ASE threshold can quantify broadband reflecting iridophores.

2. Photonic measurements

For the ASE measurements, $\sim 1 \text{ cm}^2$ sections of skin tissue were extracted from the salmon head. The mean sample thickness was $\sim 1 \text{ mm}$ and included the epidermis and dermis layers as well as some subcutaneous material. Fig. 1(a) shows an image of a salmon fish head after the rest of the animal has been processed for food. The salmon tissue was previously frozen and then thawed via standard food industry practices. Samples of skin taken from below the eyes and near the gills were viewed with an optical microscope using an adjustable-angle top-light at $\times 10$, $\times 40$, and $\times 100$ magnifications. Two sets of microscope images for different regions of the skin are also shown in Fig. 1(a). The dark silver areas of the head, which can also have a violet or blue hue were highly populated with both iridophores and melanophores. The silvery iridescent region of skin was observed to have a low surface density of melanophores while still maintaining a relatively high density of iridophores. A melanophore imaged through a backlit optical microscope and an iridophore imaged with a high-angle top-light source are shown in Fig. 1(a). Skin sections with high iridophore densities and very few melanophores were doped with a fluorescent dye. The iridescent region reflects light across the entire visible spectrum as shown in Figure S1 of the supplemental document.

Rhodamine B (RhB) was added to a 70% ethanol-water solution at a few different concentrations as shown in Figure S2. The salmon skin samples were fixed in the dye-doped solution for several days. The samples were then rinsed in a RhB/deionized water solution at 1/10th the concentration of the RhB/ethanol-water solution prior to use. After the soak, the samples were placed on a glass substrate with either the shiny epidermis face ($\mathcal{E}$ face) or dull subcutaneous face ($\mathcal{S}$ face) exposed to atmosphere.

For the ASE experiments, a vertically polarized beam of 532 nm doubled Nd:YAG laser light with a $\sim 10 \text{ ns}$ pulse width operating at 10 Hz was passed through a KG-1 filter to absorb the residual fundamental 1064 nm light. The power was reduced by passing the beam through a set of neutral density (ND) filters. A beam splitter passed a portion of the beam energy to a pyroelectric sensor, which was given a calibration factor that related the referenced pulse energy to the energy incident on the sample. The other portion of the pulse energy transmitted through the beam splitter was passed through a shutter and focused onto the sample. The other portion of the pulse energy transmitted through the beam splitter was passed through a shutter and focused onto the sample. Photobleaching of chromophores can quickly degrade the ASE

intensity [48–51], where photobleaching was minimized by (1) limiting the number of shots incident on the sample per spectrum acquisition with the shutter and (2) limiting the total number of spectra collected for each sample. A spectrometer, capped with a 550 nm long-pass filter, was used to detect the ASE signal from the sample. The long-pass filter removed the bright 532 nm pump intensity while the large Stoke's shift for RhB allows for the peak fluorescence to be captured by the detector. The peak ASE is located near the peak of the fluorescence spectrum at low concentration. The peak ASE red shifts away from the fluorescence peak for increased dye concentrations.

The emission from samples excited by the pump beam was measured using the experimental geometry shown in Fig. 1(b). The angle of the detector sweeping a horizontal plane parallel to the optical table was fixed at 15° with respect to the sample's normal direction. The pump beam spot incident on every sample had an area of $\sim 1 \text{ mm}^2$.

3. Results and discussion

The emission spectra shown in Fig. 2(a) was recorded for the $\mathcal{S}$ face of a sample stained with an ethanol-water solution containing $\sim 0.1 \text{ wt} \%$ RhB dye. The ASE spectra for the $\mathcal{E}$ face of the skin sample is shown in Fig. 2(b). The ASE recorded from the $\mathcal{E}$ face was significantly more intense than the ASE emitted from the $\mathcal{S}$ face at the same pump energy per pulse. Images of the sample's $\mathcal{E}$ face and opposing $\mathcal{S}$ face are both shown in Fig. 2(a)–(b). The ASE peak was separated from the fluorescence using a multipeak Gaussian fit of the emission intensity I as a function of the emitted photon energy E_{ph} ,

$$I(E_{\text{ph}}) = \sum_i \frac{A_i}{\sqrt{2\pi}w_i} \exp \left[-\frac{(E_{\text{ph}} - E_i)^2}{2w_i^2} \right], \quad (1)$$

where w_i is the standard deviation, A_i is the area, and E_i is the horizontal offset of the i th peak. The optimization was performed in Python with the “leastsq” function [52]. Two peaks were enough to consistently fit the spectral data; however, three-peak fits were used to more accurately assess the ASE peak area, peak position, and FWHM in some data sets. Graphs of three-peak vs. two-peak fits for a staining concentration of 0.4 wt% are shown in Figure S3. The area under the ASE peak, normalized to the largest value recorded from all samples, is shown in the upper right insets of Fig. 2(a)–(b).

ASE is often observed by first exciting a thin straight line of atoms/molecules to a relatively long-lived excited state. It can only occur when there is a population inversion with respect to a lower energy state. The threshold for ASE occurs when a single photon emitted at one end of the line induces the emission of a photon at the other end of the line. The ASE threshold in such a geometry was found to be similar to that of a traditional laser cavity, but without the effect of the cavity [53]. For a straight line, the threshold condition for ASE is given by

$$n_{\text{crit}} = \frac{8\pi \Delta\nu \tau_2}{\ell \lambda^2 \phi_B}, \quad (2)$$

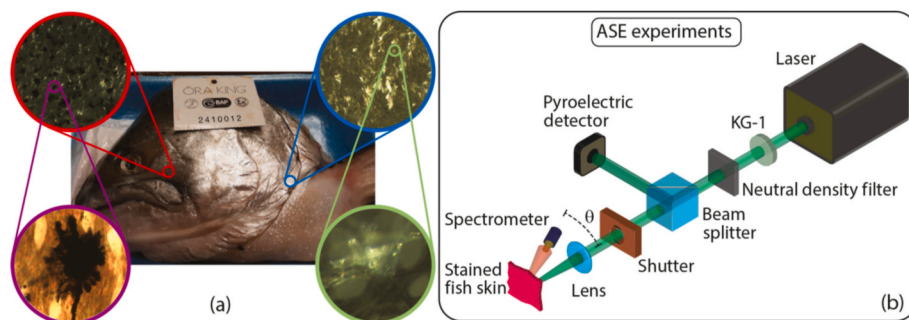


Fig. 1. (a) The head of a salmon fish with microscope images of skin tissue taken from the eye and gill regions. The skin near the eye shows both iridophores and melanophores while the skin near the gill shows a similar distribution of iridophores without the high density of melanophores. A single melanophore from the skin near the eye is shown as well as an iridophore from tissue taken near the gills. (b) The experimental setup used to investigate the ASE generation in rhodamine B-doped king salmon iridophores.

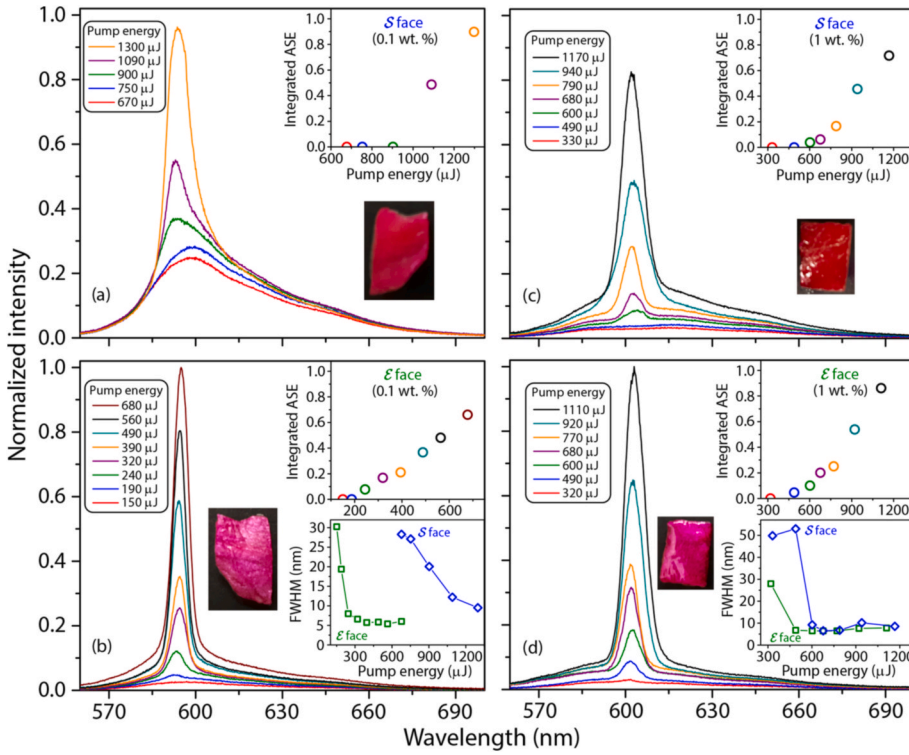


Fig. 2. The ASE spectra at various pump intensities for doped salmon skin at ~ 0.1 wt% when the sample is pumped on (a) the S face and (b) the E face. A higher stain concentration at ~ 1 wt% is shown on the right when pumped on (c) the S face and (d) the E face. An image of the sample's S and E faces are also shown in their respective spectra graphs. The ASE peak areas from multipeak fits are shown as insets in all graphs. The lower spectra graphs also display insets that plot the full-width at half-maximum (FWHM) for the ASE peak for both S and E faces at each stain concentration.

where n_{crit} is the critical population inversion density, $\Delta\nu$ is the width of the transition, ℓ is the length of the straight line, λ is the wavelength, and φ_B is the branching ratio. Note that Allen and Peters previously gave the threshold condition in terms of the line width and was nearly identical to Eq. (2) out to a factor of π [54]. The function describing the emission line is typically a convolution of Gaussian and Lorentzian functions. The ASE intensity can easily be written for the two extremes of a perfectly Lorentzian or perfectly Gaussian spectral profile. The emission intensity has since been approximated far above threshold by Svelto et al. where corrections to the Linford formula have been made above the saturation intensity, including separate factors of π for Lorentzian or Gaussian line functions [55].

A four-level model is assumed in Equation (2), where τ_2 is the lifetime of the second-excited state. The four-level model is a good approximation for laser dyes such as RhB, where a population inversion between the first- and second-excited states is easily achieved via optical pumping. Note that Equation (2) is often rewritten in terms of a critical length as opposed to the critical population inversion density due to the ease of length measurements along a single well-defined direction.

The threshold given in Equation (2) assumes a uniform population inversion density along the entire line; however, a uniform population inversion density is not always possible. Because 1) the ASE threshold is the first instance of amplification in which an emitted photon induces the emission of another photon, and because 2) the threshold condition can be written as $(\ell n)_{\text{crit}} = \text{constant}$, any heterogeneous population inversion density can be recast as a uniform one over an “effective” critical length, $(\ell_{\text{eff}} n)_{\text{crit}} = \text{constant}$. In any geometry, the ASE intensity depends on the product of the photon path length and the gain. Christianson et al. used an “effective” gain path length to describe the changes in ASE emission above the ASE threshold caused by heterogeneous photobleaching of emitters [56], where the ASE characterization resulted in the determination of self-healing properties of laser gain media that can recover after photobleaching [57].

The ASE intensity for the quasi-two-dimensional circular geometry of a thin disk laser was given as a function of an “effective” gain path length S by Peterson et al. [58]. Because the threshold criteria for ASE in a disk

laser geometry still depends on the length of a traveling photon emitted in the gain medium to induce another photon via de-excitation, then the threshold condition must be $(Sn)_{\text{crit}} = \text{constant}$. Introducing reflecting surfaces is a simple method to increase the path length of emitted photons. Many materials report low-threshold ASE, random lasing, or both in scattering media, where the density of scatterers affects the type of emission phenomenon [59]. Thus, low-threshold ASE is also anticipated for broadband-reflecting iridophores stained with an organic laser dye when there is no appreciable localization of the pump and/or emitted light.

Because the samples were submerged in the staining solution for several days, the dye concentration is assumed to be uniform throughout the sample. The lengthy soak time in the RhB/water bath results in the same aqueous environment effects for the dye molecules throughout the entire sample. Thus, the reduction of the ASE threshold for the E face relative to the S face shown in Fig. 2(a)–(b) can only be caused by the presence of the reflecting guanine platelets. Multiple reflections inside the iridophores on the iridescent skin result in an increased path length of emitted photons traveling through the gain medium.

The excited-state lifetime τ_2 is the same for RhB on either side of the skin sample. Thus, the effective gain path lengths for the S and E faces can be related at threshold,

$$\frac{S^E n_{\text{crit}}^E}{\Delta\nu^E} = \frac{S^S n_{\text{crit}}^S}{\Delta\nu^S}, \quad (3)$$

where the E and S superscripts denote the respective E and S faces onto which the pump beam is incident. Solving Equation (3) for S^E results in the expression

$$S^E = \left(\frac{n_{\text{crit}}^S}{n_{\text{crit}}^E} \right) \left(\frac{\Delta\nu^E}{\Delta\nu^S} \right) S^S. \quad (4)$$

The ASE data can be used to find a simple figure-of-merit for aperiodic structures after identifying a few simplifying terms and approximations. The ratio of the ASE line width can be determined directly from the full-width at half maximum (FWHM). When the third-excited state and first-excited state lifetimes are much shorter than the second-excited

state lifetime and there is no saturable absorption of the pump, then $n(x, y, z) \propto I_{\text{pump}}(x, y, z)$. The pump can be approximated as having a uniform intensity over the circular beam spot. The absorption length is defined as the distance the pump travels in the material until it becomes $1/e$ of its transmitted value at the bulk material interface. The gain depth is approximately equal to the absorption length, even in scattering media [60]. For the concentrations used in this study, the gain depth is shallow as compared to the dimensions of the pump beam spot incident on the surface. The pump beam on the $\mathcal{E}$ face of the sample is reflected from multiple guanine/cytoplasm interfaces at low concentration. Therefore, the gain depth for the pump incident on the $\mathcal{E}$ face is less than the gain depth for the $\mathcal{S}$ face. Also, more pulse energy is reflected out of the sample when the pump is incident on the $\mathcal{E}$ face due to the presence of the reflecting iridophores. Thus, there is an inequality $n_{\text{crit}}^{\mathcal{S}}/n_{\text{crit}}^{\mathcal{E}} \geq I_{\text{thresh}}^{\mathcal{S}}/I_{\text{thresh}}^{\mathcal{E}}$, where I_{thresh} is the threshold pump intensity incident on the sample.

A low-angle reflection measurement of the pump beam on each side of the sample was used to determine the population inversion ratio using the expression $\frac{n_{\text{crit}}^{\mathcal{S}}}{n_{\text{crit}}^{\mathcal{E}}} = \xi \frac{I_{\text{thresh}}^{\mathcal{S}}}{I_{\text{thresh}}^{\mathcal{E}}}$. The intensity being absorbed by the dye is given by $I_{\text{pump}} - I_{\text{reflect}} = I_{\text{pump}}(1 - \eta)$ with $\eta = \frac{I_{\text{reflect}}}{I_{\text{pump}}}$. Therefore the correction factor may be written as $\xi = \frac{1 - \eta^{\mathcal{S}}}{1 - \eta^{\mathcal{E}}}$, where Figure S4 shows the reflected intensities for a pump beam incident on both sides of a sample stained with a 0.1 wt% RhB solution. For a concentration of 0.1 wt%, the effective gain path length of an emitted photon through the $\mathcal{E}$ face with exposed iridophores is approximately 5.6 times longer than the effective gain path length when pumped on the $\mathcal{S}$ face, i.e., $S^{\mathcal{E}} \approx 5.6 S^{\mathcal{S}}$.

Using the ratio of effective gain path lengths as a figure-of-merit requires careful consideration of the dopant concentration. As the RhB concentration in the staining solution increases, the absorption depth of the pump decreases. A high concentration RhB solution such as 1 wt% results in a pump beam incident on the $\mathcal{E}$ face reaching fewer platelet interfaces. Thus, the intensity of the reflected beam approaches the reflected intensity when the pump is incident on the $\mathcal{S}$ face for the same pump intensity. Although a greater amount of energy per pulse is absorbed by the dye, the gain depth decreases and the gain medium becomes shallow. When the excited-state population dwindles in the region where multiple guanine/cytoplasm reflections occur, the effective gain path length of the $\mathcal{E}$ face approaches that of the $\mathcal{S}$ face. Fig. 2 (c)–(d) shows the emission spectra at various pump intensities for a skin sample stained by a 1 wt% RhB solution. The threshold pump intensities for ASE are comparatively closer between the $\mathcal{S}$ face and $\mathcal{E}$ face illumination, $I_{\text{thresh}}^{\mathcal{S}} \approx 1.2 I_{\text{thresh}}^{\mathcal{E}}$, as compared to the thresholds observed for samples stained with weaker RhB concentrations. The spectral widths are also observed to be much closer at greater stain concentrations as compared to those stained with weaker concentrations. The peak ASE wavelength is the same when pumped on either face of a sample for a given stain concentration. The peak ASE wavelength on either side redshifts when stained with greater RhB concentrations. Note that the sample burn threshold was quickly reached after passing the ASE threshold on the $\mathcal{S}$ face of the 1 wt% sample, which limited the upper range for both the concentration and pump intensity in this study. Laser damage to biological tissue from the absorption of visible light by dyes and pigments is well documented [61–64].

Fig. 3 shows the concentration dependence of the ASE threshold when pumped on the $\mathcal{S}$ and $\mathcal{E}$ faces. The stark increase in the ASE threshold as a function of concentration when pumped on the $\mathcal{E}$ face occurs after increasing the staining concentration above 0.1 wt% RhB. There is a slight decrease in the ASE threshold at increased staining concentrations when the samples were pumped on the $\mathcal{S}$ face. When the RhB concentrations increase, the mechanisms of reduced quantum yield and increased emitter density compete which result in a relatively small ASE threshold reduction over the experimental range of concentrations.

The pump beam absorption depth decreases as the concentration increases. Because the diameter of the beam spot is much greater than

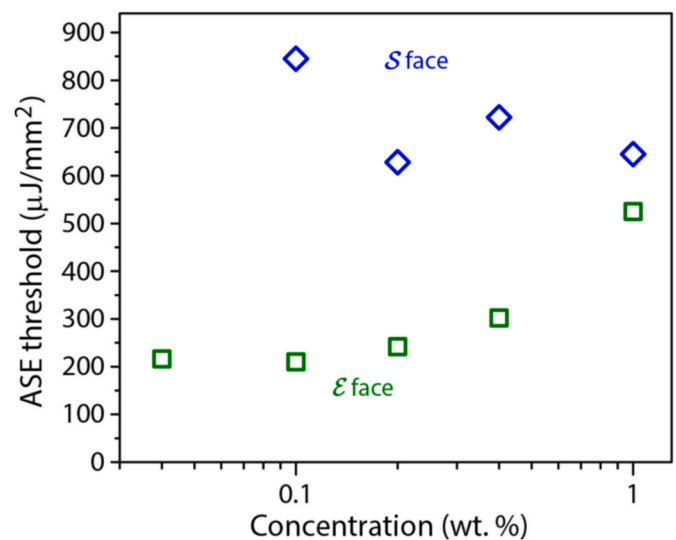


Fig. 3. The ASE threshold as a function of RhB dye concentration in the ethanol staining/fixing bath for salmon skin illuminated on either the $\mathcal{S}$ or $\mathcal{E}$ face.

the gain depth for the RhB concentrations used in this study, the dependence of the ASE threshold on the gain depth is negligible when the pump beam is incident on the $\mathcal{S}$ face. When the pump beam is incident on the $\mathcal{E}$ face, however, the absorption depth determines the extent of pump light absorption in the iridophore cells. As the gain depth becomes sufficiently short at high concentrations, the pump does not penetrate into the guanine/cytoplasm layers and the threshold of the $\mathcal{E}$ face approaches the value observed when pumped on the $\mathcal{S}$ face. Note that the relative ASE threshold for the $\mathcal{E}$ is significantly lower than the $\mathcal{S}$ for the 0.1 wt% concentration, but the absolute values are relatively high as compared to some studies. These relatively high ASE thresholds are primarily due to the lower quantum efficiency of RhB in aqueous solution relative to other solvents such as methanol, longer pump pulse duration used in this experiment, and the pump beam geometry being circular as opposed to a long gain strip.

When multiple dielectric layers with alternating high/low refractive indices are periodically distributed, constructive interference causes certain colors to be reflected. A lack of any definite color in the reflected light over a small surface area is a result of there being no well-defined reflection band over a small patch of illuminated surface. Multilayer structures with (1) large random thickness variations, (2) well-defined chirped layers, and (3) groupings of small layered clusters with angular and thickness variations can all result in the far-field, broadband, reflection spectrum observed in Figure S1. All possible broadband-reflecting structures formed by guanine crystal platelets and cytoplasm can increase a photon's path length after being emitted from a fluorescent chromophore in the cytoplasm.

As stated, there are three types of structures that could occur in iridophores which would result in the broadband reflection spectrum observed in Figure S1. If a material is composed of small clusters of periodic structures, then lasing should occur in small localized regions. Broad reflection bands with low dispersion can be observed if the reflecting structures are either chirped or have chaotically distributed layer thickness. Lasing in a localized region is improbable in these one-dimensional aperiodic structures because of the high group velocities associated with the low dispersion of the reflector. Lasing was not observed in any of the samples pumped with a circular spot at high intensity, which led to the conclusion that the photonic structure was not composed of small clusters of periodic multilayers.

Thus, the layers must either be chirped or chaotically distributed. There has been recent interest in aperiodic photonic structure in both fabrication methods and the broadband enhancement of optical phenomena [65–71]. The transmission electron microscope (TEM) images

in Fig. 4 show aperiodic photonic structures with chaotic distributions of guanine platelets in the cytoplasm of iridophores. The guanine platelet thicknesses are relatively constant with large variations in the thicknesses of the cytoplasm layers. Because silvery fish skin is often a byproduct of the food industry, it can be used as a low-cost alternative to many aperiodic photonic structures that have more costly fabrication methods. These structures also have a liquid layer which can be used to modify their photonic properties; the liquid layer can also be polymerized to create an entirely solid-state aperiodic photonic structure as was done for the TEM analysis and shown in Figure S5. The technique for TEM sample preparation is given in TEM Methods found after Section 4.

The broadband reflecting iridophores show little dispersion in the

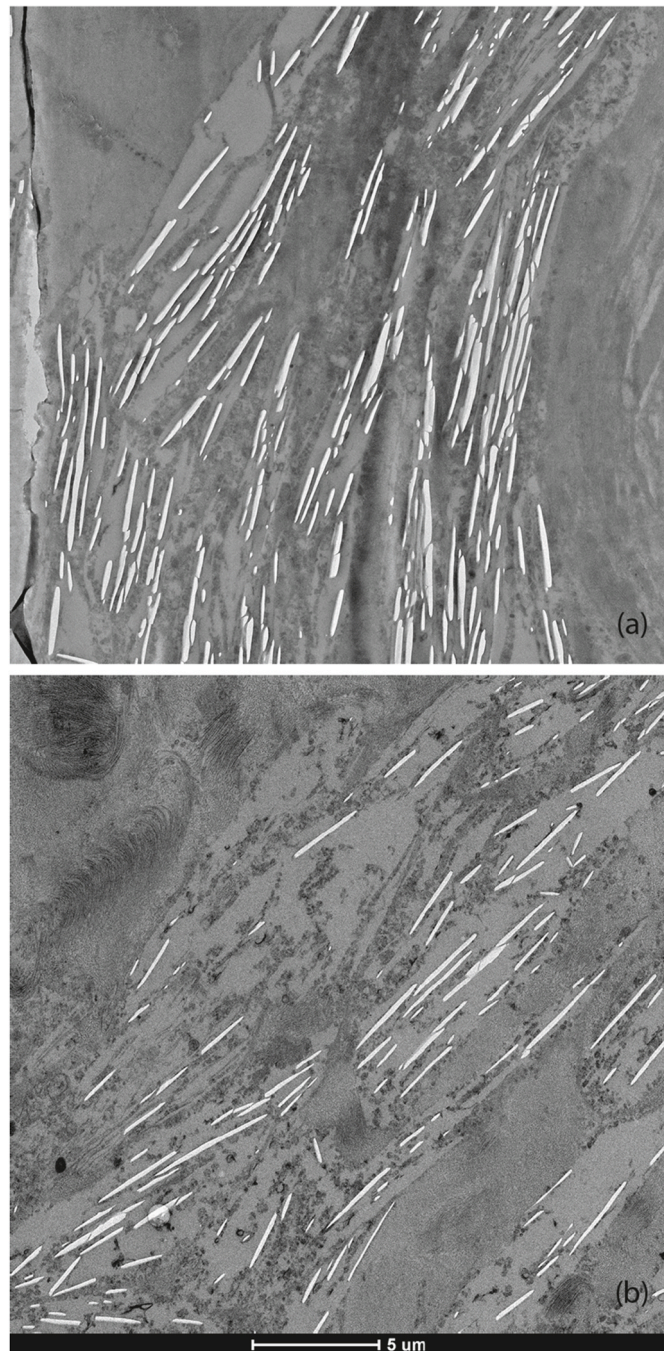


Fig. 4. Transmission electron microscope images of transverse sectioned skin samples showing an (a) aperiodic distribution and a (b) sparse distribution of guanine platelets. The scale bar represents a distance of 5 μm .

visible spectrum. Therefore, the observation of reduced-threshold ASE was not accompanied by low-threshold lasing. What appeared to be high-threshold lasing was briefly observed in one sample stained with a high concentration of RhB, but only after a coverslip was placed over the sample and the pump beam was shaped into a line. Short-range reflections inside the iridophores coupled with the long-range guiding of light emission via high-angle reflections at the coverslip interfaces were attributed to the coherent backscattering that resulted in random lasing from the ASE profile. The emission spectrum in Figure S6 shows a bi-modal ASE profile with multiple random lasing peaks emerging, where the energy per pulse of the pump laser illuminating the thin strip caused the sample to burn. Unlike the ASE experiments, lasing in samples using a coverslip and line pump profile was not easily reproduced.

Several optical techniques have already been developed to measure the reflective properties of iridophores. This paper presents a novel photonic technique to measure the path length of photons in fish skin with silvery iridescence. The plethora of fish species with iridophores having various photonic and optical properties could result in new photonic materials and devices, where new methods of characterizing these biomaterials may prove important. There were some limitations in this study such as sample-to-sample variation in the surface density of iridophores, the specimens were frozen/thawed, and the skin was dehydrated by the ethanol vehicle in the staining procedure. In addition, the pump wavelength was limited to 532 nm and the active molecule in the gain medium was limited to RhB. It would be interesting to characterize the ASE emitted at shorter and longer wavelengths to determine how the broadband reflecting iridophores affect the ASE over a broader range of emission wavelengths. More studies on other species will be necessary to investigate the breadth of the photonic biodiversity of pelagic teleosts and the effective ASE gain path length's merit in determining a broadband-reflecting iridophore's capacity as a photonic biomaterial.

4. Conclusion

The ASE threshold was significantly reduced in the skin of king salmon when photons were emitted from iridophores stained with a fluorescent laser dye. At a staining concentration of 0.1 wt% RhB in a 70% ethanol-water solution, the pump was able to substantially penetrate into the iridophore-containing skin surface. The presence of the iridophores on the E face reduced the ASE threshold as compared to the ASE measured from the S face. The effective gain path length in silvery king salmon skin samples was estimated to be 5.6 times greater than the stained subcutaneous tissue. The dependence on stain concentration further confirmed that the reduced-threshold ASE was due to gain in the iridophores. It has been suggested that Anderson localization can occur in silvery fish iridophores [72]. The lack of low-threshold random laser emission using the pumping scheme shown in Fig. 1 is indicative of delocalized light over the entire gain envelope of RhB; however, results could differ for fresh samples with the use of a cell-permeating fluorescent dye and without the use of a nonaqueous vehicle. The absence of random laser light emission without the aid of an external glass reflector further suggests that even weak photon localization was negligible under these experimental conditions. Thus, the increase in the emitted-photon path lengths through the gain medium is the dominant mechanism for the reduced ASE thresholds being reported.

5. TEM methods

The samples were fixed in a modified Karnovsky's solution with 2% paraformaldehyde (PFA), 2.5% glutaraldehyde (GA), and 0.1 M phosphate buffer and transported to the Franceschi Microscopy and Imaging Center at Washington State University. After arrival, samples were transferred into a 0.1 M phosphate buffer on cutting board. Each sample was cut down to $\sim 1 \text{ mm}^3$, then placed back in the 2% PFA, 2.5% GA, and 0.1 M phosphate buffer at 4 $^{\circ}\text{C}$ overnight. Samples were rinsed the

following day three times ($3 \times$), 10 min each, with 0.1 M phosphate buffer. The samples were then placed in 1% osmium tetroxide overnight at 4 °C. After post-fixing with osmium, the samples were rinsed as before and then dehydrated through an ethanol series (30%, 50%, 70%, 95%, and 100%- $3 \times$) for 10 min each step. Samples were incubated in 100% propylene oxide (PO) for 10 min, then infiltrated with propylene oxide: Spurr's resin at 2:1 overnight at room temperature. The following morning the infiltration solution was increased to 1:1. The solution was replaced with 100% Spurr's resin at the end of the day and left overnight at room temperature. After two more changes of 100% Spurr's resin, samples were polymerized in molds overnight at 70 °C. Thin sections were cut using glass knives on a Reichert-Jung ultramicrotome. The sections were picked up on a formvar coated slot grid, stained with 2% uranyl acetate for 8 min, and then Reynolds lead for 8 min. Samples were observed on an FEI T20 TEM using a 200 kV accelerating voltage.

Author statement

Nathan J. Dawson: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing. Valerie Lynch-Holm: Methodology, Investigation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jlum.2021.118474>.

References

- [1] J.W. Hawkes, The structure of fish skin, *Cell Tissue Res.* 149 (1974) 159–172, <https://doi.org/10.1007/BF00222271>.
- [2] D. Gur, E.J. Bain, K.R. Johnson, A.J. Aman, H.A. Pasoili, J.D. Flynn, M.C. Allen, D. D. Deheyn, J.C. Lee, J. Lippincott-Schwartz, D.M. Parichy, In situ differentiation of iridophore crystalloids underlies zebrafish stripe patterning, *Nat. Commun.* 11 (1) (2020) 6391, <https://doi.org/10.1038/s41467-020-20088-1>.
- [3] D.M. Parichy, Evolution of pigment cells and patterns: recent insights from teleost fishes, *Curr. Opin. Genet. Dev.* 69 (2021) 88–96.
- [4] M. Iwasaka, Flashing spots on the dorsal trunk of hardyhead silverside fish, *R. Soc. Open Sci.* 8 (4) (2021) 201578, <https://doi.org/10.1098/rsos.201578>.
- [5] D. Gur, B.A. Palmer, S. Weiner, L. Addadi, Light manipulation by guanine crystals in organisms: biogenic scatterers, mirrors, multilayer reflectors and photonic crystals, *Adv. Funct. Mater.* 27 (6) (2017) 1603514, <https://doi.org/10.1002/adfm.201603514>.
- [6] H. Wakabayashi, M. Asai, J. Yamakita, Three-dimensional analysis of structural coloration by a slanted dielectric grating, *J. Opt. Soc. Am. A* 37 (10) (2020) 1539–1547, <https://doi.org/10.1364/JOSA.A.394683>.
- [7] P.C. Brady, K.A. Travis, T. Maginnis, M.E. Cummings, Polaro-cryptic mirror of the lookdown as a biological model for open ocean camouflage, *Proc. Natl. Acad. Sci. Unit. States Am.* 110 (24) (2013) 9764.
- [8] P.C. Brady, A.A. Gilerson, G.W. Kattawar, J.M. Sullivan, M.S. Twardowski, H. M. Dierssen, M. Gao, K. Travis, R.I. Etheredge, A. Tonizzo, A. Ibrahim, C. Carrizo, Y. Gu, B.J. Russell, K. Mislinski, S. Zhao, M.E. Cummings, Open-ocean fish reveal an omnidirectional solution to camouflage in polarized environments, *Science* 350 (6263) (2015) 965–969, <https://doi.org/10.1126/science.125284>.
- [9] P.C. Brady, A.A. Gilerson, G.W. Kattawar, J.M. Sullivan, M.S. Twardowski, H. M. Dierssen, M. Gao, K. Travis, R.I. Etheredge, A. Tonizzo, A. Ibrahim, C. Carrizo, Y. Gu, B.J. Russell, K. Mislinski, S. Zhao, M.E. Cummings, Open-ocean fish reveal an omnidirectional solution to camouflage in polarized environments, *Science* 350 (6263) (2015) 965–969.
- [10] L.M. Mäthger, M.F. Land, U.E. Siebeck, N.J. Marshall, Rapid colour changes in multilayer reflecting stripes in the paradise whiptail, *pentapodus paradiseus*, *J. Exp. Biol.* 206 (20) (2003) 3607–3613.
- [11] T. Jordan, J. Partridge, N. Roberts, Non-polarizing broadband multilayer reflectors in fish, *Nat. Photonics* 6 (2012) 759–763.
- [12] S. Zhao, P.C. Brady, M. Gao, R.I. Etheredge, G.W. Kattawar, M.E. Cummings, Broadband and polarization reflectors in the lookdown, *selene vomer*, *J. R. Soc. Interface* 12 (104) (2015) 20141390.
- [13] M. Gather, S. Yun, Single-cell biological lasers, *Nat. Photonics* 5 (2011) 406–412.
- [14] A. Jonás, M. Aas, Y. Karadag, S. Maniöglu, S. Anand, D. McGloin, H. Bayraktar, A. Kiraz, In vitro and in vivo biolasing of fluorescent proteins suspended in liquid microdroplet cavities, *Lab Chip* 14 (16) (2014) 3093–3100.
- [15] S. Nizamoglu, K.-B. Lee, M.C. Gather, K.S. Kim, M. Jeon, S. Kim, M. Humar, S.-H. Yun, A simple approach to biological single-cell lasers via intracellular dyes, *Adv. Opt. Mater.* 3 (9) (2015) 1197–1200, <https://doi.org/10.1002/adom.201500144>.
- [16] M. Karl, C.P. Dietrich, M. Schubert, I.D.W. Samuel, G.A. Turnbull, M.C. Gather, Single cell induced optical confinement in biological lasers, *J. Phys. D Appl. Phys.* 50 (8) (2017), 084005, <https://doi.org/10.1088/1361-6463/aa5367>.
- [17] D. Septiadi, V. Barna, D. Saxena, R. Sapienza, D. Genovese, L. De Cola, Biolasing from individual cells in a low-q resonator enables spectral fingerprinting, *Adv. Opt. Mater.* 8 (6) (2020) 1901573.
- [18] M. Schubert, A. Steude, P. Liehm, N.M. Kronenberg, M. Karl, E.C. Campbell, S. J. Powis, M.C. Gather, Lasing within live cells containing intracellular optical microresonators for barcode-type cell tagging and tracking, *Nano Lett.* 15 (8) (2015) 5647–5652, <https://doi.org/10.1021/acs.nanolett.5b02491>.
- [19] M. Humar, S.H. Yun, Intracellular microlasers, *Nature photon* 9 (9) (2015) 572–576.
- [20] J. Mysliwiec, K. Cyprych, L. Sznitko, A. Miniewicz, Biomaterials in light amplification, *J. Opt.* 19 (3) (2017), 033003.
- [21] Y.-C. Chen, X. Fan, Biological lasers for biomedical applications, *Adv. Opt. Mater.* 7 (17) (2019) 1900377, <https://doi.org/10.1002/adom.201900377>.
- [22] Y.-C. Chen, Q. Chen, T. Zhang, W. Wang, X. Fan, Versatile tissue lasers based on high-q fabry-pérot microcavities, *Lab Chip* 17 (3) (2017) 538–548.
- [23] M. Umar, K. Min, S. Kim, S. Kim, Random lasing and amplified spontaneous emission from silk inverse opals: optical gain enhancement via protein scatterers, *Sci. Rep.* 9 (1) (2019), 16266–16266.
- [24] H.H. Mai, T.T. Nguyen, K.M. Giang, X.T. Do, T.T. Nguyen, H.C. Hoang, V.D. Ta, Chicken albumen-based whispering gallery mode microlasers, *Soft Matter* 16 (2020) 9069–9073, <https://doi.org/10.1039/D0SM01091J>.
- [25] S.-W. Chen, J.-Y. Lu, B.-Y. Hung, M. Chiesa, P.-H. Tung, J.-H. Lin, T.C.-K. Yang, Random lasers from photonic crystal wings of butterfly and moth for speckle-free imaging, *Opt Express* 29 (2) (2021) 2065–2076, <https://doi.org/10.1364/OE.414334>.
- [26] S.-W. Chen, J.-Y. Lu, P.-H. Tung, J.-H. Lin, M. Chiesa, B.-Y. Hung, T.C.-K. Yang, Study of laser actions by bird's feathers with photonic crystals, *Sci. Rep.* 11 (1) (2021), 2430–2430.
- [27] R.C. Polson, Z.V. Vardeny, Random lasing in human tissues, *Appl. Phys. Lett.* 85 (7) (2004) 1289–1291.
- [28] P. Vukusic, J.R. Sambles, C.R. Lawrence, R.J. Wootton, Quantified interference and diffraction in single morpho butterfly scales, *Proc. R. Soc. Lond. B Biol. Sci.* 266 (1427) (1999) 1403–1411.
- [29] M.A. Giraldo, S. Yoshioka, D.G. Stavenga, Far field scattering pattern of differently structured butterfly scales, *J. Comp. Physiol.* 194 (3) (2008) 201–207.
- [30] S. Kinoshita, S. Yoshioka, J. Miyazaki, Physics of structural colors, *Rep. Prog. Phys.* 71 (7) (2008), 076401.
- [31] J.M. Haag, J.S. Jaffe, A.M. Sweeney, Measurement system for marine animal reflectance functions, *Opt Express* 21 (3) (2013) 3603–3616.
- [32] A. Ghoshal, D.G. DeMartini, E. Eck, D.E. Morse, Experimental determination of refractive index of condensed reflectin in squid iridocytes, *J. R. Soc. Interface* 11 (95) (2014) 20140106.
- [33] M.D. Cahn, A.C. Brown, E.D. Clotfelter, Guanine-based structural coloration as an indicator of oxidative stress in a cichlid fish, *J. Exp. Zool. A Ecol. Genet. Physiol.* 323 (6) (2015) 359–367.
- [34] N.J. Dawson, K.D. Singer, J.H. Andrews, M. Crescimanno, G. Mao, J.B. Petrus, H. Song, E. Baer, Post-process tunability of folded one-dimensional all-polymer photonic crystal microcavity lasers, *Nonlin. Opt. Quant. Opt.* 45 (1–2) (2012) 101–111.
- [35] J.H. Andrews, M. Aviles, M. Crescimanno, N.J. Dawson, A. Mazzocco, J.B. Petrus, K.D. Singer, E. Baer, H. Song, Thermo-spectral study of all-polymer multilayer lasers, *Opt. Mater. Express* 3 (8) (2013) 1152–1160.
- [36] M. Humar, M.C. Gather, S.-H. Yun, Cellular dye lasers: lasing thresholds and sensing in a planar resonator, *Opt Express* 23 (21) (2015) 27865–27879, <https://doi.org/10.1364/OE.23.027865>.
- [37] A. Mohamadi, H. Poorantiyosh, Amplified spontaneous emission (ase) effect on gain and stored energy, *J. Opt.* 49 (2020) 469–475.
- [38] Y. Kawabe, L. Wang, S. Horinouchi, N. Ogata, Amplified spontaneous emission from fluorescent-dye-doped dna-surfactant complex films, *Adv. Mater.* 12 (17) (2000) 1281–1283.
- [39] J. Mysliwiec, L. Sznitko, S. Bartkiewicz, A. Miniewicz, Z. Essaidi, F. Kajzar, B. Sahraoui, Amplified spontaneous emission in the spiropyran-biopolymer based system, *Appl. Phys. Lett.* 94 (24) (2009) 241106.
- [40] J. Mysliwiec, L. Sznitko, A. Miniewicz, F. Kajzar, B. Sahraoui, Study of the amplified spontaneous emission in a dye-doped biopolymer-based material, *J. Phys. D Appl. Phys.* 42 (8) (2009), 085101.

- [41] L. Sznitko, J. Mysliwiec, P. Karpinski, K. Palewska, K. Parafiniuk, S. Bartkiewicz, I. Rau, F. Kajzar, A. Miniewicz, Biopolymer based system doped with nonlinear optical dye as a medium for amplified spontaneous emission and lasing, *Appl. Phys. Lett.* 99 (3) (2011), 031107.
- [42] I. Rau, A. Szukalski, L. Sznitko, A. Miniewicz, S. Bartkiewicz, F. Kajzar, B. Sahraoui, J. Mysliwiec, Amplified spontaneous emission of rhodamine 6g embedded in pure deoxyribonucleic acid, *Appl. Phys. Lett.* 101 (17) (2012) 171113, <https://doi.org/10.1063/1.4764535>.
- [43] Y.-C. Hung, C.-H. Su, H.-W. Huang, Low threshold amplified spontaneous emission from dye-doped dna biopolymer, *J. Appl. Phys.* 111 (11) (2012) 113107.
- [44] F. Lahoz, C.J. Oton, D. López, J. Marrero-Alonso, A. Boto, M. Díaz, High efficiency amplified spontaneous emission from a fluorescent anticancer drug-dye complex, *Org. Electron.* 14 (5) (2013) 1225–1230, <https://doi.org/10.1016/j.orgel.2013.02.015>.
- [45] C. Pradeep, C.P.G. Vallabhan, P. Radhakrishnan, V.P.N. Nampoori, Amplified spontaneous emission from picogreen dye intercalated in deoxyribonucleic acid lipid complex, *Laser Phys. Lett.* 12 (12) (2015) 125802.
- [46] N.M. Hoinka, T. Fuhrmann-Lieker, Amplified spontaneous emission in paper, *Sci. Rep.* 9 (2019) 1862.
- [47] K.A. Christensen, J.S. Leong, D. Sakhrani, C.A. Biagi, D.R. Minkley, R.E. Withler, E. B. Rondeau, B.F. Koop, R.H. Devlin, Chinook salmon (*Oncorhynchus tshawytscha*) genome and transcriptome, *PLoS One* 13 (4) (2018), e0195461.
- [48] B.F. Howell, M.G. Kuzyk, Amplified spontaneous emission and recoverable photodegradation in polymer doped with disperse orange 11, *J. Opt. Soc. Am. B* 19 (8) (2002) 1790–1793, <https://doi.org/10.1364/JOSAB.19.001790>.
- [49] N.B. Embaye, S.K. Ramini, M.G. Kuzyk, Mechanisms of reversible photodegradation in disperse orange 11 dye doped in pmma polymer, *J. Chem. Phys.* 129 (5) (2008), 054504, <https://doi.org/10.1063/1.2963502>.
- [50] S.K. Ramini, N. Dawson, M.G. Kuzyk, Testing the diffusion hypothesis as a mechanism of self-healing in disperse orange 11 doped in poly(methyl methacrylate), *J. Opt. Soc. Am. B* 28 (10) (2011) 2408–2412.
- [51] N. Stubbs, M. Bridgewater, M. Stubbs, M. Crescimanno, M.G. Kuzyk, N.J. Dawson, Polylactic acid promotes healing of photodegraded disperse orange 11 molecules, *Opt. Mater.* 76 (2018) 11–15.
- [52] `scipy.optimize.leastsq` – `scipy` v1.6.1 reference guide. <https://docs.scipy.org/doc/scipy/reference/generated/scipy.optimize.leastsq.html>. (Accessed 25 October 2020).
- [53] G.I. Peters, L. Allen, Amplified spontaneous emission i. the threshold condition, *J. Phys. A* 4 (2) (1971) 238–243, <https://doi.org/10.1088/0305-4470/4/2/009>.
- [54] L. Allen, G.I. Peters, Superradiance, coherence brightening and amplified spontaneous emission, *Phys. Lett.* 31 (3) (1970) 95–96.
- [55] O. Svelto, S. Taccheo, C. Svelto, Analysis of amplified spontaneous emission: some corrections to the linford formula, *Opt Commun.* 149 (4) (1998) 277–282.
- [56] N.D. Christianson, Y. Lu, N.J. Dawson, Recovery of photodegraded rhodamine 6g in ester-containing polymer matrices, *Photochem. Photobiol. Sci.* 18 (12) (2019) 2865–2874, <https://doi.org/10.1039/C9PP00243J>.
- [57] Y. Lu, Z. Lowther, N.D. Christianson, Z. Li, E. Baer, M.G. Kuzyk, N.J. Dawson, Demonstration of a self-healing all-polymer distributed bragg reflector laser, *Appl. Phys. Lett.* 116 (10) (2020) 103301, <https://doi.org/10.1063/1.5145148>.
- [58] P. Peterson, A. Gavrielides, T.C. Newell, N. Vretnar, W.P. Latham, Ase in thin disk lasers: theory and experiment, *Opt Express* 19 (25) (2011) 25672–25684.
- [59] H. Cao, J.Y. Xu, S.-H. Chang, S.T. Ho, Transition from amplified spontaneous emission to laser action in strongly scattering media, *Phys. Rev. E* 61 (2) (2000) 1985–1989, <https://doi.org/10.1103/PhysRevE.61.1985>.
- [60] H. Cao, Lasing in random media, *Waves Random Media* 13 (3) (2003) R1–R39, <https://doi.org/10.1088/0959-7174/13/3/201>.
- [61] D.J. Goldberg, Laser removal of pigmented and vascular lesions, *J. Cosmet. Dermatol.* 5 (3) (2006) 204–209.
- [62] F. Pinto, S. Große-Büning, S. Karsai, C. Weiß, W. Bäuml, S. Hammes, M. Felcht, C. Raulin, Neodymium-doped yttrium aluminium garnet (nd:yag) 1064-nm picosecond laser vs. nd:yag 1064-nm nanosecond laser in tattoo removal: a randomized controlled single-blind clinical trial, *Br. J. Dermatol.* 176 (2) (2017) 457–464.
- [63] M. Hohmann, D. Dörner, F. Mehari, C. Chen, M. Späth, S. Müller, H. Albrecht, F. Klämpfl, M. Schmidt, Investigation of random lasing as a feedback mechanism for tissue differentiation during laser surgery, *Biomed. Opt Express* 10 (2) (2019) 807–816, <https://doi.org/10.1364/BOE.10.000807>.
- [64] E. Austin, A.N. Geisler, J. Nguyen, I. Kohli, I. Hamzavi, H.W. Lim, J. Jagdeo, Visible light. part i: properties and cutaneous effects of visible light, *JAAD* 84 (5) (2021) 1219–1231, <https://doi.org/10.1016/j.jaad.2021.02.048>.
- [65] M. Bellingeri, A. Chiasera, I. Krieger, F. Scotognella, Optical properties of periodic, quasi-periodic, and disordered one-dimensional photonic structures, *Opt. Mater.* 72 (2017) 403–421, <https://doi.org/10.1016/j.optmat.2017.06.033>.
- [66] U.G. Yasa, Z.G. Figen, H. Kurt, Photonic crystals based on aperiodic dielectric multilayers for optical filtering, *J. Opt.* 21 (4) (2019), 045101, <https://doi.org/10.1088/2040-8986/ab06d1>.
- [67] J. Wu, Y. Chen, Broadband radiative cooling and decoration for passively dissipated portable electronic devices by aperiodic photonic multilayers, *Ann. Phys.* 532 (5) (2020) 2000001, <https://doi.org/10.1002/andp.202000001>.
- [68] N.-M. Wang, H. Huang, C. Zhang, Broadband second harmonic generation in aperiodic nonlinear photonic crystals: 1d projection from 2d vogel sunflower spiral array, *AIP Adv.* 11 (5) (2021), 055219, <https://doi.org/10.1063/5.0052800>.
- [69] F. Sgrignuoli, Y. Chen, S. Gorsky, W.A. Britton, L. Dal Negro, Optical rogue waves in multifractal photonic arrays, *Phys. Rev. B* 103 (2021) 195403, <https://doi.org/10.1103/PhysRevB.103.195403>.
- [70] F. Getman, M. Makarenko, A. Burguete-Lopez, A. Fratallocchi, Broadband vectorial ultrathin optics with experimental efficiency up to 99% in the visible region via universal approximators, *Light Sci. Appl.* 10 (1) (2021) 47.
- [71] N.B. Ali, Y. Trabelsi, H. Alsaif, Y. Bouazzi, M. Kanzari, Optimized omnidirectional high-reflectance using octonacci photonic crystal for thermographic sensing applications, *Photonics* 8 (5) (2021) 169, <https://doi.org/10.3390/photonics8050169>.
- [72] T.M. Jordan, J.C. Partridge, N.W. Roberts, Disordered animal multilayer reflectors and the localization of light, *J. R. Soc. Interface* 11 (101) (2014) 20140948.