Bio-inspired optics: general discussion

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Andrew Parnell opened a general discussion of the paper by Giuseppe M. Paternò: All bacteria are negatively charged, so will the positive charge of the silver not just pull any of the planktonic bacteria to the surface differentially and just cause a coating? You are not measuring the kinetics/process of biofilm formation as you are forcing them to inhabit/coat the silver surface. Also, are the bacteria on the surface dead or alive? Have you tried optically imaging them with a live/dead optical stain?

Giuseppe Maria Paternò responded: Thanks for your question. Yes, I think this scenario would be realistic. For dead/live count, I guess you mean classical staining methods. In this case, we haven’t done anything like this yet. Thanks for the suggestions.

Christian Kuttner enquired: Has the stability of the layered stack in agar/LB been investigated over longer periods of time? One might expect that the media could penetrate the layered structure. Is this the case, and if so, how would this affect the optical properties?

Giuseppe Maria Paternò replied: Thanks for your question. The aqueous culture medium does infiltrate the porous structure. This leads to a red-shift of the photonic band gap. Usually, this occurs a few minutes after the exposure.

Alex Qiu asked: Can the sensor be reset by cleaning the bacterial culture from it?

Giuseppe Maria Paternò answered: Thanks for your question. Full reversibility cannot be achieved, mostly because of silver degradation upon interaction with bacteria. We have data showing that bacteria affect both the morphological integrity and crystallinity of the silver layer.
Amanda Holt queried: Would gold provide different functionalities? Also, could the sensor be designed in a reflectance style, and how would that affect the sensitivity?

Giuseppe Maria Paternò responded: Thanks for your question. It is known that gold is also capable of interaction with bacteria. We are currently also investigating this metal. We anticipate that we would see a less obvious modulation of its optical properties upon interaction with bacteria, compared to silver.

Stefano Fornasaro asked: Can you comment on the selectivity of the sensor? Does the response change with different bacteria?

Giuseppe Maria Paternò replied: Thanks for your question. We are studying the effect upon exposure to Gram-positive bacteria, *Micrococcus luteus* (*M. luteus*). This bacterium is of interest in the field of food safety, as it has recently been found in contaminated chicken. In this case, we could not observe strong indications of silver uptake by *M. luteus* cells, as in the case of *E. coli*. In addition, we did not observe the strong photonic shift that occurs for Gram-negative bacteria. Therefore, we found that our approach is in principle more sensitive to Gram-negative than Gram-positive bacteria, a result that we attribute to the more effective electrostatic interaction and cellular adhesion between the negatively charged outer membrane of Gram-negative bacteria and the positively charged silver ions. Please see our paper for more detail (DOI: 10.1039/d0fd00026d).

Giselle Rosetta enquired: Do you foresee these sensors being used for many different bacteria at the same time, for example the dual detection of *E. coli* and *Listeria*?

Giuseppe Maria Paternò responded: Thanks for your question. At the moment, we cannot envision any discrimination of different bacteria in a complex environment. To achieve this, we are currently working on another approach, namely the exploitation of Tamm optical states at the interface between a photonic crystal and a plasmon metal. We would like to modulate this electromagnetic field enhancement upon interaction with specific biochemical functionalities (*i.e.* antibodies) to be able to interact selectively with different bacterial species.

Sébastien R. Mouchet asked: Could other biological entities such as viruses be detected with such hybrid plasmonic/photonic 1D crystals, too?

Giuseppe Maria Paternò replied: Thanks for your question. Silver has also been proven to be active against several types of viruses, including human immunodeficiency virus, hepatitis B virus, herpes simplex virus, respiratory syncytial virus, and monkey pox virus. We haven't investigated the possible effects of such interactions on the optical properties of silver, thus we cannot say whether this could also be translated into a photonic read-out. We reckon that this could be an interesting field of investigation.

Christian Kuttner queried: Could it be possible that the change in optical properties is related to chemical changes (*e.g.*, the oxidation of Ag)?
Giuseppe Maria Paternò answered: Thanks for your question. Yes, this could indeed be a possibility, *i.e.* oxidative dissolution of silver when exposed to bacteria. See for instance ref. 1 and 2.


Andrew Parnell asked: Is there free silver in the system? If we added an organic layer to the surface of the silver like a charged micelle would we get the same colour response? Basically, can the sensor discriminate?

Giuseppe Maria Paternò responded: Thanks for your question. Our samples consist of a silver layer obtained *via* thermal evaporation (8 nm). The film morphology is rather discontinuous, consisting of an ensemble of silver nanoparticles with average diameter 15–45 nm. We’ve also exposed these silver samples to liposomes composed of *E. coli* lipid extract, noting also in this case a blue-shift of the plasmon absorption.

Christian Kuttner opened a general discussion of the paper by Ullrich Steiner: Could you please briefly explain (for non-experts) the meaning of the Cauchy principal value and how it can be evaluated?

Ullrich Steiner replied: The Cauchy principal value is a finite number that can be attributed to an integral over a function that has a divergence in the integration range. The approach is to split up the integration into parts that can be integrated and compare their divergence at the discontinuity. The standard example is the integration of $1/x$ symmetrically around the origin, where it has a divergence, say between $-1$ and $1$. Since the divergence of $1/x$ is symmetrical around the origin, the areas under $1/x$ with $x$ approaching zero from $-1$ and from $1$ are identical but of opposing sign. The principal value of this integral is therefore zero.

Christian Kuttner commented: You mentioned the “crosstalk” between $\kappa$ and $n$ described by the Kramers–Kronig relations. Could you briefly describe why this is the case and how this has been respected in the calculations of the refractive indices?

Ullrich Steiner responded: What I mean by “crosstalk” is the fact that when using the complex notation of the complex refractive index $n = n + i\kappa$, $n(\lambda)$ and $\kappa(\lambda)$ are not independent, but linked. The functional relationship between the two is given by the Kramers–Kronig relation in eqn (2) of the article, which can be used to calculate the spectral variation of the refractive index if $\kappa(\lambda)$ is known. There is also an inverse relation allowing the calculation of $\kappa(\lambda)$ for a known variation of $n(\lambda)$. There is a fundamental underpinning this relation that is connected to the causality of light propagation in matter. There is considerable literature on this aspect of the KK relations (which is unfortunately not easy to digest).¹

Ming Xiao remarked: It’s a smart way to use the absorption of the pigments to enhance the refractive index, which can offer many possibilities. How about the effect of the broad-band absorber? For example, is the high refractive index of melanin also caused by its absorption? In addition, are there any physical limitations/assumptions of the Kramers–Kronig relations? Can they be applied to any type of material?

Ullrich Steiner replied: As illustrated in the model calculations in Fig. 1, the $\Delta n$ enhancement stems mainly from sharp gradients in $\kappa$ at wavelengths that are shorter than that where the maximum $\Delta n$ is observed. The plateau area of a broad-band pigment contributes little to the effect, mainly spreading out the increase in refractive index in the wavelength range towards its maximum.

Concerning the high refractive index of melanin, see Fig. 3c in ref. 1. The melanin refractive index has a distinct Cauchy-like variation (eqn (5) in the paper) in the visible range. This means that the high refractive index stems from features in the melanin absorption spectrum in the UV (much smaller than 400 nm).

Concerning your last two questions, the KK relations are fundamental, stemming from the causality principle in light scattering and the finiteness of the speed of light propagating through dielectric media. They apply to all linear optical media.


Bianca Datta asked: What are the limitations of the dyes you used? Do you have plans to test any others?

Ullrich Steiner answered: There are three limitations: (1) the peak absorption coefficient of the dye; (2) the steepness of the gradient of absorption decay towards long wavelengths; (3) the maximum solubility in the carrier medium. As indicated in our outlook slide, we believe that all three aspects can be improved by carefully selecting other dyes, and yes, we are planning to investigate this.

Amanda Holt enquired: Have you tried using these dyes in any of the applications mentioned in a prototypical way, and what would be the primary limitations?

Ullrich Steiner responded: Not yet. For organic dyes bleaching may be an issue, particularly for high intensity illumination.

Helen Clark asked: I am curious – please would you explain how you ensure homogeneity of the dye concentration in your experiments? Is it important to maintain a constant temperature?

Ullrich Steiner replied: We mainly check whether the dye dissolves well and does not sediment out. The solutions have to be clear – light scattering is an indication of dye aggregation. The refractive index is temperature dependent, but compared to the measured KK variation of $\Delta(n)$, the temperature dependent refractive index change of water $\pm 5 ^{\circ}C$ around room temperature is negligible.
(\leq 10^{-3})$. It is therefore sufficient to carry out the experiments at room temperature without further temperature stabilisation.

**Lukas Schertel** addressed Ullrich Steiner and Bodo Wilts: Given that the absorption has to be relatively large to influence the real part of the refractive index considerably, is the macroscopic optical appearance of the materials governed by the absorption, or is there an optimal regime where the real part of the refractive index is strongly influenced but the signal is not dominated by absorption?

**Bodo Wilts** responded: Thank you for the interesting question. Whether this effect happens strongly depends on the absorption profile. As can be seen from our contribution (particularly Fig. 1), the increase of the real part of the refractive index can also be significant far away from the absorbance band and result in a significant increase of the real part of the refractive index in a region where the material is transparent.

**Ullrich Steiner** responded: The stronger the decay of \( \kappa \) towards long wavelengths, the lower the overlap of the absorption spectrum with the spectral domain in which \( \Delta n \) has its maximum. This is illustrated in Fig. 3b in our article. If one is interested in the refractive index in a narrow-band spectral domain (e.g. for laser optics), it is therefore possible to have a very strongly enhanced refractive index and nearly no absorption.

The “macroscopic optical appearance” is perhaps a different matter. If part of the absorption spectrum lies at visible wavelengths (as is the case for all dyes in our study), then yes, the appearance of the medium is affected by both absorption and refraction. If, however, finite absorption lies entirely in the UV (just outside the visible range), as for example in the white butterfly in ref. 1, then the effect of the enhanced refractive index is observable without a superposed absorption effect.


**Thomas G. Parton** commented: The effect of the dye absorption \( \kappa \) on the real component of the refractive index is quite striking in your work using commercial dyes. Would you expect natural pigments to substantially change the dispersion of the refractive index? I’m thinking of chlorophyll in cells as a particular example. Accounting for the absorption of these pigments is important for modelling the optical response of cells, but I haven’t seen work also accounting for the absorption effect on the real refractive index component.

**Ullrich Steiner** replied: Yes, natural pigments can significantly change the refractive index, as demonstrated in ref. 16 in our paper. Chlorophyll is an interesting case. Both chlorophyll A and B have very high extinction coefficients between 400 and 450 nm, with sharp decays towards long wavelengths. This should enhance the refractive index around 500 nm. To check this in living cells is presumably not easy, but chlorophyll can be commercially obtained and dissolved in a suitable solvent (e.g. an alcohol) to test this.
Alex Qiu asked: Have you experimented with combining multiple dyes in solution and checking how well the KK relations relate the absorption and refractive index? In particular, do you know if the fitted Cauchy-type contribution of the dye is straightforwardly additive on combination?

Ullrich Steiner answered: Yes, differentiation Cauchy contributions are additive. This is the basis of Fig. 4 in our article. In her MSc thesis, Tianqi Sai has also experimented with dye mixtures. These data have unfortunately not found their way into our article.

Laura Ospina remarked: Since estimating the imaginary part of the RI is difficult for biological structures, we sometimes consider only the real part. How much do you think this can affect our conclusions of the relationship between structures and the visual effects they produce? Is there any mathematical approach we could use to overcome this problem?

Ullrich Steiner responded: This is an intrinsic problem plaguing all optical systems. The KK relations allow us to predict the “real” refractive index if the entire extinction spectrum is known at lower wavelengths (down to 0 nm). This is of course unrealistic for any experimental system. Our article attempts to give an answer to how to circumvent this conundrum by fitting the refractive index variation far from absorption features in the spectrum by a Cauchy relation (which has of course been known for a long time). The paper also demonstrates how to deal with optical spectra where there is an interplay between absorption and refraction in the special range of interest.

Christian Kuttner commented: Your approach for determining the effective refractive index would also be very useful for other research fields that work with liquids of intense colours. For instance, colloidal dispersions of plasmonic nanoparticles\(^1\) and their superstructures\(^2\)–\(^4\) could yield quite interesting effective refractive indices. Are there additional effects that first need to be taken into account before applications of your approach?


Ullrich Steiner replied: Indeed, the use of nanoparticle suspensions should be interesting, since the high extinction coefficients of solid materials could be harnessed. Concerning plasmonic nanoparticles: the plasmon resonance depends sensitively on the dielectric surroundings of the plasmonic emitter. This could be tuned using the KK approach.
Christian Kuttner noted: Prof. Steiner pointed out that the Kramers–Kronig approach allows for a hypothetical pigment with a sharply defined boxcar-shaped absorption band. Can such an ideal pigment be found in nature or any artificial material? If I understand correctly, such a pigment would in principle act like a notch filter.

Ullrich Steiner answered: I doubt that a boxcar-shaped absorber can be found. Fig. 1 merely serves to illustrate the functional variation of $\Delta n$ as a function of $\kappa(\lambda)$. It might, however, be possible to mix several absorbers to approximate a boxcar-shaped absorption profile.

Stefano Fornasaro opened a general discussion of the paper by Radislav Potyrailo: How did you select the number of principal components for the PCA? How did you validate the results?

Radislav Potyrailo responded: Mathematically and conventionally the number of principal components (PCs) is selected based on the trend in the relative values of their contributions – you look for a change in the slope of such a trend and pick the smaller number. From an experimental perspective, conventionally the number of PCs is selected based on how many gases you use for testing. An ideal instrument should have as many PCs as the number of tested gases if the goal is to discriminate all gases.

Bianca Datta asked: By using machine learning tools, how different were the structures and features you were able to generate in comparison to existing design rules or previously known structures?

Radislav Potyrailo answered: At this point we were optimizing existing general geometries. In future, we will go more “wild” or broad.

Christian Kuttner commented: It appears that the dynamic response is much more pronounced for $\text{H}_2$ than for CO (see Fig. 11). Is this the case, and if so, why?

Radislav Potyrailo replied: The dynamic response is typically affected by several factors, including the catalytic reaction kinetics of different gases with metal nanoparticles of different sizes and different metals. We believe that this factor may be one of the dominant ones.

Bianca Datta enquired: How quickly does the response occur and how repeatable is the response?

Radislav Potyrailo responded: In our DARPA program, a response was shown in less than 1 s. The response time is strongly affected (convoluted) by the flow rate of the tested gas and the volume of a gas flow cell. The repeatability error is a few percent and typically increases with time in any analytical instrument.

Mike Hardy remarked: Thank you for the fantastic presentation, Radislav. Can you comment, generally speaking, on how close bio-inspired multivariable sensors can get to single-output sensors in terms of cost, size, portability, etc.?
Radislav Potyrailo answered: We calculated that 2 mm x 2 mm chips can be 40 cents each.

Helen Clark asked: You say in your paper that low power use is a desired factor in your work. Are you intending to eventually implement neuromorphic computing methods for gas discrimination as well as the computational machine learning methods you describe? Neuromorphic chips are designed for low power use, and I’ve seen some interesting reports of smell recognition.¹


Radislav Potyrailo replied: Thank you for the reference to the Intel/Cornell paper.¹ We are indeed developing methodologies for on-board data analytics. In our paper, Table 1 shows examples of typical machine learning (a.k.a. multivariate analysis, chemometrics) tools for data analysis of multivariable sensors. The next version of this table in our future reviews will include neuromorphic and other emerging methods. The final selection of the method will be based on the size of the calibration sets, the results of field validations, and other parameters such as power, etc.


Christian Kuttner commented: In Fig. 3d of your contribution, it is difficult to see where the Au nanoparticles are located – could you please clarify this? Is the distribution of Au homogeneous or do the particles form larger aggregates in the layers?

Radislav Potyrailo answered: The gold nanoparticles (Au NPs) are sandwiched between the two layers of ceria. The underlayer of ceria is deposited first, then gold with dewetting into NPs, then the overcoat of ceria is deposited. The gold layer can be seen in this image on the top lamella layer as it has the largest amount of area to coat. The Au NPs are specifically sandwiched between the two layers of ceria.

Sébastien R. Mouchet enquired: In Fig. 3b of your paper (and the fourth slide of your talk), you show some examples of 3D photonic structures inspired by *Morpho* wing scales. They seem to have square shapes when observed from the top. However, *Morpho* wing scales do not display square shapes, but ridges. I imagine the squares were designed to improve the sensing properties. Wouldn’t disk shapes be more advantageous from a detection point of view? However, I would expect them to be more complicated to synthesise. Am I right to think so?

Radislav Potyrailo replied: We show them as squares because of our etching masks.

Stefano Fornasaro asked: What information did you get from the loadings? Is there a reason for not showing them?
Radislav Potyrailo answered: Loadings show how different independent variables (wavelengths in our sensor) contribute to different principal components. Sometimes loadings are shown in the supplementary information; they are less interesting to look at. People rarely show them as main figures in published papers.

Gea Theodora van de Kerkhof opened a general discussion of the paper by Franziska Schenk: In nature you can find many different photonic structures, each with different optical effects. How well can you fine-tune the exact angle at which light is reflected from a surface, using currently commercially available effect pigments? Can you get any angle you want by choosing the right product?

Franziska Schenk responded: I am slightly confused by this question. What is meant by “any angle you want”? Does this refer to the reflectance colour and/or the angle at which maximum reflection is achieved? Regarding the latter, just as in nature, the multi-layered effect pigments available display maximum reflectance at the face angle (i.e. perpendicular to the surface) and shift towards the shorter wavelength/s at oblique angles. In fact, this shift to a shorter wavelength with increasing angle of light incidence is an inherent property of reflecting (multi-)layers in general.

As regards achieving varied iridescent colour effects, there is a range of different effect pigment families currently available, each with distinctively different optical signatures. Some are narrow-band reflectors such as those detailed in this paper, while others display broader-ranging colour-travel. Therefore, while perhaps there are not as many different kinds as in nature, a multitude of different shades and effects can be achieved by inter-mixing within the same flake family, and also across different flake types, as well as with pigments.

Laura Ospina commented: I am really curious about the application process of the flake pigment. Do you have to ensure a very thin layer of this material? Do you need to ensure a particular orientation and order of the flakes? What is the interaction between the flakes and the pigments underneath?

Franziska Schenk replied: The flake pigment does indeed have to be applied in very thin layers. To intensify the reflection colour multiple thin layers can be added. When suspended in a suitable liquid binder and applied with experience and precision, the flakes self-orientate parallel to the substrate surface. As regards the rules of interaction between the flakes and pigments underneath, these are still poorly understood, and more research is needed – as clearly arriving at ‘mixing’ rules that combine ‘subtractive’ and ‘additive’ light mixtures is of crucial interest to both artists and scientists alike. The case most readily explained (and cited in the literature) is when black pigment is located beneath the reflector. In this instance, all non-reflected light waves pass through and are fully absorbed by the black – a very pure reflection colour results. However, more complex cases of pigment-flake interaction are in urgent need of further investigation.
Leila Deravi enquired: Can you comment on the interactions happening between the flakes in the suspension you are creating and speak to the formulation design some more?

Franziska Schenk answered: If suspended in a water-soluble transparent liquid binder and brushed/sprayed on carefully in one direction, the flakes self-orientate and align with the substrate's surface. However, it requires extensive practice to master the best approach.

Thomas G. Parton asked: Have you tested whether these structural pigments are more resistant to UV degradation? Pigment fading is a common issue for old artwork.

Franziska Schenk replied: The structural materials in question are 100% UV resistant. They do not fade when exposed to sunlight like traditional pigments do. Only when their nanoarchitecture is destroyed by applying too much pressure will they stop reflecting colour. Million-year-old fossil specimens have been unearthed that still display their iridescent colouration (while the pigmentary layer once located beneath will have faded away).

Amanda Holt queried: Have you measured any optical properties of the final artwork? Does the final iridescent color application require multiple “coats”?

Franziska Schenk answered: We have measured the angle-dependent reflectance of the final artwork, which matches that of the specimen convincingly. Indeed, the final iridescent colour application required multiple “coats” to achieve the desired colour intensity.

Christian Kuttner remarked: In your contribution you present very beautiful examples of art. For the A. ilia wing, are the intense colours mainly due to the scattering of the light or does the absorption of the light also play a role?

Franziska Schenk responded: The effect is due to a combination of absorption and light scattering. The painting mimics what happens on the wings of A. ilia. A pigmented ground is overlaid with multi-layered interference pigments analogous to the reflectors present in the butterfly's cover scales. In both systems the top layer reflects light in the blue-UV region only, while all other light waves pass through to the pigmented layer beneath – the latter selectively absorbing/scattering the remaining light components, depending on the particular nature of the pigment in question.

Sébastien R. Mouchet commented: I really enjoyed reading your paper. Very nice painting, too. I am always amazed by your work. Thanks for this. In your paper, you wrote that it aims to lead to “smarter” art. Based on the optical effects of Apatura sp. (violet to UV iridescence – “now you see it, now you don’t”), could you comment further on what sort of artistic effects could be developed (for other kinds of painting)? Maybe some sort of new “trompe-l’œil”?
Franziska Schenk replied: This is a most pertinent question, as clearly it is a new “trompe-l’œil” that I am after, with the current work only being the first stage. I am planning to develop future paintings that, for example, combine a range of violet shades that appear identical to the naked eye, but differ in terms of their UV-reflectivity. This being a true case of “now you see it, now you don’t”, an unexpected pattern/image will emerge temporarily when viewed through a UV-filter.

Helen Clark remarked: The paintings are beautiful – what is the way forward that you envisage for the technique? Will I be able to buy/make the coating to replicate the effect?

Franziska Schenk answered: Some of the effect pigments that we investigated (i.e. those developed by Merck) are available as ready-made artists’ paint now. However, of the types we used for the final artwork one has been discontinued (i.e. LCP Helicone® Sapphire) due to problems with fragility when used for certain high-pressure printing applications. The other, Firemist® Violet, like many comparable special effect pigment families, still remains mainly restricted to industrial usage – the main reason being that these specialist optical materials are often classified and for military and security applications only. However, such limitations/restrictions are now in the process of being overcome. No doubt, as the technology continues to advance, both the materials themselves, as well as the specialist knowledge needed to apply them to full effect, will become more readily accessible.

Carlos Fiorentino addressed Franziska Schenk and Doekele Stavenga: Thank you for your presentation. Your work is very interesting, and the only one at the conference involving art, so I’m glad I was part of it (I’m a visual communication designer myself). One of the slides of your presentation mentioned that your project follows principles of biomimetic design, learning from nature. It is my understanding that the reason for nature to “come up” with structural colour “solutions” may be that blue pigment is relatively scarce and hard to afford in nature. So we can speculate that when a needed colour (e.g. bright blue) cannot be afforded by pigment, nature “tries” structural colour, evolving from subtractive to additive mixing of light or combining both. So in the case of the painting, if the structural colour effect is achieved by a pigment base mixed with materials producing structural colour to mimic what is observed in the butterfly… Would this strategy be the opposite of what nature does? Like reverse engineering nature? What is the lesson learned from nature?

Franziska Schenk responded: The lesson learned from nature is that diverse strategies have evolved over millions of years that successfully combine – what we (not nature) understand as – two distinctly different colour systems/theories. Combining both, in turn, results in the widest possible portfolio of optical effects. In nature this is a matter of survival – and perhaps it is in art, too? In any case, the tenet is that studying these very strategies in nature can help artists devise analogous creative methods.

Also, as an aside, the assumption that in terms of evolution, pigments (subtractive colour mixing) came before structural colour (additive colour mixing)
is debatable. The ‘two systems’ may well have co-evolved. Hence perhaps our ultimate aim ought to be the conception of one united colour system/theory that incorporates both pigment and structural colour.

**Doekele Stavenga** responded: As is often the case, the situation is not black or white. Plant nature has lots of examples of blue due to pigment, and some animals also feature blue pigments. On the other hand, examples of plants coloured by photonics are rapidly accumulating. My uneducated guess is that plants, being sessile, cannot exploit the striking display effects of iridescent structures, which can be very effective in communication for mobile animals. We know of many cases where structural colouration is improved by combining it with a pigment-based selective band filter. Concerning Franziska’s paintings, an underlayer of black pigment is adequate to let the iridescent effect-pigment stand out. A brown pigment will add a brown tinge. (I have to ask her if she ever combined the effect-pigments with a wavelength selective filtering pigment.) The uniqueness of her work is that she can not only reproduce butterflies at much larger magnitudes, but that the onlooker, when observing the painting from different positions, gets to see the same colour changes as happen with the real thing, adding a strong dynamic aspect.

**Conflicts of interest**

There are no conflicts to declare.