nature portfolio

Peer Review File

Heart cockle shells transmit sunlight to photosymbiotic algae using bundled fiber optic cables and condensing lenses

Corresponding Author: Dr Dakota McCoy

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The paper by Johnsen et al. reports on the optical properties of the cockle shells. It is claimed that the aragonitic fibrous crystals that appear as bundles, serve as fiber optic cables and condensing lenses.
There are several examples in biomineralization where calcium carbonate serves as part of an optical device. One example is the brittle star as shown by Prof. Aizenberg of Harvard U.
In this case though teh authors paint an interesting story that is only supported by simulations and not proved experimentally as compared to the work of Aizenberg et al. Due to this serious flaw the paper remains a possibility or a plausible scenario but is not sufficiently sound to be published in a general audience journal such as nature comm.
It is true for example that the c-axis of aragonite is more transmitting then the a-axis. And true that if one adds more a-axis it is more scattered. but this yet does not prove this is the actual biological function in the shell!
There are many examples that shells have beautiful c-axis oriented calcite prisms (the only optical axis that has no birefringence and simulations may show it is an excellent optical transmitter, and yet in practice it has no optical function.
The proof of biological function is clearly lacking in this paper.
In addition I also find he paper not recorder and there is a mix-up in the figures:
Page 4: I think there's a mistake in the referral.. Figure 4A is, and i quote, "Polished shell fragment shows the fibrousprisms rotating in orientation between the regions 288 labeled 1-3, such that in region 3 we are looking at a cross sections of aligned prisms pointing into the page."
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Page 6: again.... Figure 3B is a schematic of the arrangement.. it is definitely not a "grayscale image encoding height information"
and again, they even say that it is (and I quote): "Figure 3: Condensing lenses with truncated caps under the windows focus sunlight. By
“truncated”, we refer to the flattened tips of the lens. (A-B) A cross section of a heart cockle shell shows that beneath each shell window in some shells, the aragonite forms a lens shape roughly 750 µm in diameter. The exact size and shape of the lens varies from shell to shell.
226 Beneath the shell is soft tissue with photosynthetic symbionts."
Page 8: and again... they state that 4B is polished lens SEM image in the figure description.. something went wrong with their numbering...

Reviewer #2

(Remarks to the Author)
The manuscript, "Heart cockle shells transmit sunlight for photosynthesis using bundled fiber optic cables and condensing lenses", is interesting, well-written and deserves to be published in Nature Communications as is. I do have some minor considerations for the authors outlined below.

This paper is an intiguing analysis of the novel biophotonic system in heart cockle shells that has relevance to other photo-symbiotic systems within molluscs and extends to other more in vogue systems like reef building corals. The data clearly shows the optical outcomes of the unique morpology, ie focusing light and screening higher energy harmful radiation. I appreciate the depth of the optical analysis in defining this unique system.

In Figure 2 A-D it would be nice to include the illumination images of the sand-facing side of the shells with the same light intesity and image exposure as the portrayed sun-facing sides. This would help achieve the goal of this portion of the figure in giving more of a contextual feel of the system to the reader.

The references sections of Figure 3 from the main text and the caption to need to be reviewed and updated. Example: line 230 "J-L" there is no "L" in the figure, line 256 "Figure 4 E-G" is probably supposed to be "F-H", etc. Check these all these references for accuracy.

If there are no limitations to figure counts, I would reccommend that Supplemental Figure 3 is moved to the main body of the paper. Since this is a relatively novel system I think that it is necessary to demonstrate that the material is aragonite as opposed to another form of calcium carbonate or other mineral, S3 is the most compelling data to show this.

Overall the paper is highly publishable and interesting. Congratulations on some fine work.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have made efforts to improve the manuscript and have added several experiments. The most important addition is to show no dependence of transmission on thickness.
The biological function is yet missing and all that is presented in this respect is speculation. Without proving there is a real biological function meas the concept simply dies on the vine.

Reviewer #3

(Remarks to the Author)
In the paper, “Heart cockle shells transmit sunlight for photosynthesis using bundled fiber optic cables and condensing lenses,” McCoy et al. describes, models and measures the way light passes through the sun-facing shell (in contrast to the sand-facing shell), through a potentially condensing lens and into the algae-rich portion of the animal. The authors expand on the existing theories that the windows allow the bivalve to mechanically protect its soft tissue while letting beneficial light pass through to feed the photosymbionts. As previously shown, the windows are made from the same materials as the shell only organized/crystallized into bundles of elongated spires. The authors expand on this knowledge by exploring through microscopy, how the cockle shell’s windows project images and by modeling how differently sized fibers result in more or less transmission. The authors also find that some of the windows have bumps on the inside of the shell and explore the existing idea that these may serve as lenses and find they have focal distances along the order of where it has been shown that most of the photosymbionts are located.

While this work mostly expands on existing observations and theories, the experiments are creative and informative and models are new. This paper however, touches the surface of many of these ideas without going into the details I would like to see and leaves me wondering more rather than understanding comprehensively how the system is working or how evolution led to this point (or both). This aspect could be improved by either focusing in on a particular optical piece of the system or by viewing the system as a whole and showing all the interactions and how they lead to the most desired result. The following are more specific comments that could help in either direction.

- I don’t fully grasp the idea that these are the first “fiber optic cable bundles” .This is stated in the abstract and in the results section. What constitutes a bundle? I understand that there are few to many instances of fiber optic cables. Are the bundles simply that they are organized into smallish shapes - the windows - and not consistent throughout? Or, is actually that within the windows, there are separate bundles of fibers? And, why would they need/use these fiber optics fibers in the form of bundles if they are within the window?

- The above leads me to another comment: More information on average sizes of the windows, fibers and bundles is needed as well as their extent in the z direction along with variance would be informative. This information is hard to glean from any of the pictures or microscope images. I also think a more detailed image or drawing with increased magnification of the windows , fibers and bundles would help one “visualize” the system - like Figure 6b.

- In the Results section on the transparent windows, the authors state they cut flat 1 cm^2 fragments. What does flat mean in this context? Meaning there isn’t must variation in shell thickness at 1 cm^2 areas? What is the typical shell thickness where the windows are and are the windows the same thickness as the shell aside from the lenses?

- in relation to the above, in the supplemental information, the authors show how shell thickness does not correlate with transmission which is hard to wrap my head around. Is this because the measurements are taken with an integrating sphere so only absorption would cause a decrease in transmission, where there isn’t much? Also, the polished shells at 300 microns thick do show a significantly increased transmittance. Would that be because they are polished? If one were measuring only transmittance (without an integrating sphere), I would expect that scattering (and absorption) would effect how much light is transmitted through, therefore a thicker, scattering material would result in less transmittance than a thinner one. Clarity on this should be in the manuscript especially since the sun-facing shell is overall thinner than the sand-facing shell and that thickness and optical thickness in optics is an important and informative parameter. I bet someone has measured the scattering and absorption coefficients of bivalve shells in general and the authors should cite this and understand it.

- Also, what wavelength is Supplemental figure s7 measured at? If measured in the UV, would you see a difference?

- Can the authors cite the reference to absorption and scattering of Calcium Carbonate? And find a reference that shows absorption and scattering of similarly composed shells.

- Also, what physical/optical aspect about the windowed side allows statistically more visible light than UV light when compared to the sand-facing side? The mention of Calcium carbonate is true for both sides of the shell. (Though I can't find where shells scatter light like 1/lambda - can the authors cite that?) This is again a section where shell thickness is a natural first guess and perhaps scattering and absorption is non-linear with wavelength resulting in nonlinear changes in transmittance. But, if it is the windows and how they are designed, that would be interesting and relevant and the authors should show/describe it.

- In Figure 2, the grey and black lines are mislabeled in the caption.

- in the Condensing lenses section, the authors mention they found some individuals with lenses. Why only some individuals? Does that mean this aspect is not as important? And, were the lenses found on all the windows or only in a specific portion of the shell?

- The authors show that the lenses/bumps focus light to around 1 mm with modeling and experiment. They discuss how this may be to reach the symbionts but in order to solidify this argument, more evidence beyond what is cited would be helpful. Is there any way for the authors themselves to experimentally show the location of symbionts and how the rest of the tissue is organized the lenses?

- question from a non-expert: Are the micro lenses and windows evolved from eyes?

- in the lens simulation, it would be nice to have a depth in addition to a scale bar. Also, why doesn't the simulation extend beyond the depth shown? It would also be interesting to know where the focal point ends.

- It's neat that the optical fiber bundles project images, but what would the significance of this be biologically? And/or can you use this observation to make quantitative or characteristic determinations about the underlying structure? Showing this is fine and neat but making it more relevant to the biology or to measurement is necessary.

- The paragraph on Anderson localization is also interesting but mostly descriptive and the authors provide no evidence that this system transmits light by that effect.

- The authors make a statement that since the transmission spectra do not show noticeable absorption peaks, the UV radiation must be screened primarily by imperfections in the fiber optic structures. While it's true that there are no peaks (except for the colorful shells) the significant drop in UV for both shells indicates absorption (and scattering) especially since this measurement is with an integrating sphere. Unless there is significantly more UV reflection or back scattering - which the authors would have to show to make this claim.

- If the authors put the shell inside an integrating sphere, they could measure exactly the absorption.

- The authors state that 1 micron diameter fibers is superior to small fiber diameters in the visible and only slightly inferior. By looking at their plot, I would argue that larger diameters result in significantly higher transmittance. Would there be a mechanistic reasoning why the bivalve would not want even higher transmission?

Version 2:

Reviewer comments:

Reviewer #3

(Remarks to the Author)
The authors have addressed most or all of my concerns and as a result, I think their paper is more comprehensive and informative.

One mistake that I noticed is that in figure S9, part (a) states: "The sun-facing sides of shells are significantly thicker than the sand-facing sides of shells". I believe it should be: "The sun-facing sides of shells are significantly thinner than the sand-facing sides of shells."

On line 165, you mention screening blue light and UV radiation but there is no mention of blue light for the rest of the paper. My first thought when reading that is that blue light is between 400-500 nm and is spectrally where algae absorb the most light so why would they want to screen "blue" light. It's nuanced but my advice is to just stick to UV in verbiage.

Otherwise, great job improving the paper's impact.

<u>Response to Reviewer Comments</u>

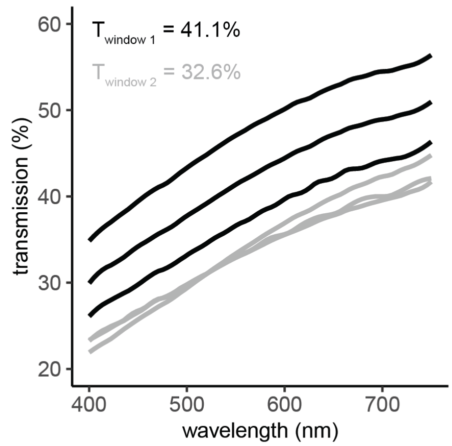
Reviewer #1 (Remarks to the Author):

The paper by Johnsen et al. reports on the optical properties of the cockle shells. It is claimed that the aragonitic fibrous crystals that appear as bundles, serve as fiber optic cables and condensing lenses.

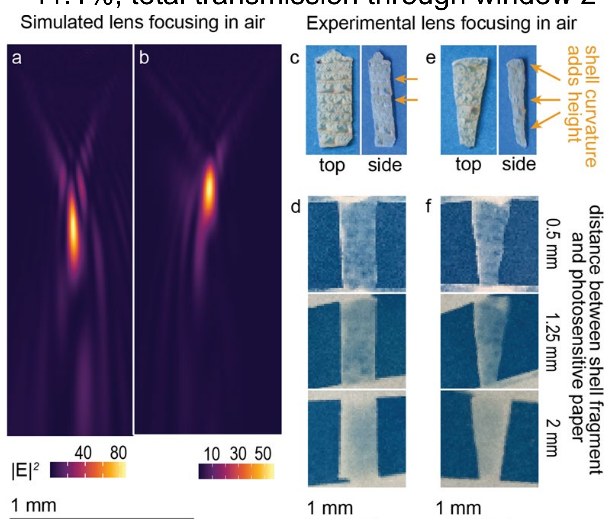
There are several examples in biomineralization where calcium carbonate serves as part of an optical device. One example is the brittle star as shown by Prof. Aizenberg of Harvard U.

In this case though teh authors paint an interesting story that is only supported by simulations and not proved experimentally as compared to the work of Aizenberg et al. Due to this serious flaw the paper remains a possibility or a plausible scenario but is not sufficiently sound to be published in a general audience journal such as nature comm.

- **We appreciate the comment that further experimental procedures could strengthen the paper—and our new experiments have greatly improved the manuscript. Therefore, we added two new experimental analyses, following Aizenberg et al., as described below.**
- **The reviewer mentions that we only support our work by simulations, rather than experiments, but in fact experiments comprise about half of our paper (see Figure 2, Figure 4, and Figure 5 as well as panels in the other figures). We know the excellent work of Aizenberg et al, and like their work, we experimentally measure light transmission and optical properties of the material (actual heart cockle shells collected from wild populations).**
- **Our two new experimental analyses are:**
    - **First, we measure transmission through individual shell windows polished to a thickness of 300 micrometers in order to ground truth our simulations. The findings are consistent with the values from our simulations. validating our approach.**
    - **Second, we performed experimental validation that the lenses focus light.**
    - **The new figures are copied below; see also the main text for revisions to the methods and results.**



- **Supplemental Figure S1. Individual polished shell windows transmit 32.4%-46.3% of light (median=34.5%).** We polished individual shell windows to a width of 300 µm, so that we could ground-truth our simulations of light transmission (Main text, Figure 5). We measured transmission through the shell windows at three locations within each of two windows. Total transmission through window 1 = 41.1%; total transmission through window 2 = 32.6%.



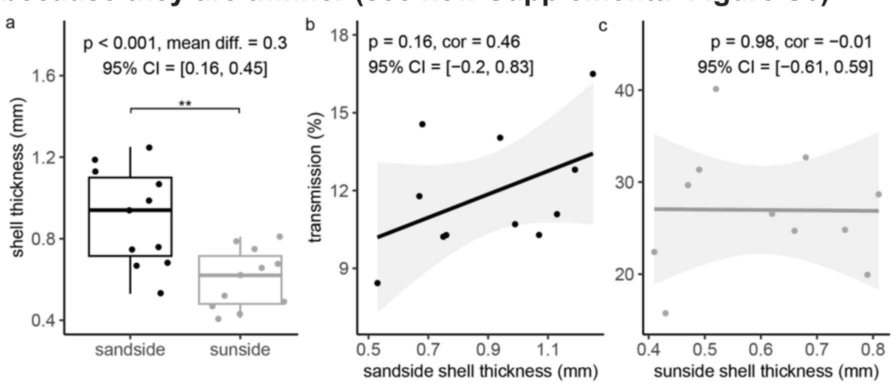
- **Supplemental Figure S3. Microlenses focus light in experimental validation of simulations.** We used photosensitive paper to create imprints of solar irradiation at different depths beneath shell fragments; these experiments were done in air using natural sunlight. **(a-b)** We repeated our Lumerical simulations of lens focusing with air as the background medium. The most intense region of focus typically ranged from 0.5-1 mm below the shell surface. **(c-f)** We placed a shell fragment atop photosensitive cyanotype paper to visualize the lensing effect of light focusing and dispersing (**d** and **f**; top to bottom) at three depths: 0.5mm, 1.25mm, and 2 mm. However, these depths are not absolute, because the shell fragments had some natural curvature **(c**, **e**: orange arrows) that added roughly 0.25-0.75 mm of height. The depths reported in **d-f** were measured from the lowest points on the shell fragments.

- **Finally, we absolutely agree with the reviewer that biological function must be demonstrated, not just hypothesized. We added a new section of text directly addressing this point, titled "*Determining biological function from optical properties".***

It is true for example that the c-axis of aragonite is more transmitting then the a-axis. And true that if one adds more a-axis it is more scattered. but this yet does not prove this is the actual biological function in the shell! There are many examples that shells have beautiful c-axis oriented calcite prisms (the only optical axis that has no birefringence and simulations may show it is an excellent optical transmitter, and yet in practice it has no optical function. The proof of biological function is clearly lacking in this paper.

- **This is an excellent point: just because a structure in nature has optical properties does not mean that those properties are involved in the biological function. We strongly appreciate and agree with this perspective. We have added the following text to convey this point but also make the case that the optical properties in heart cockles serve a clear purpose:**
  - ***Determining biological function from optical properties***
  - Just because a natural material has certain optical properties does not mean that those optical properties serve a biological purpose to the organism. Marvelous photonic properties may occur as a side effect of selection for a different purpose, such as toughness or smoothness. For example, blind golden moles (Chrysochloridae) have iridescent green, purple, and golden hairs arising from thin-film interference [64]. But the moles are blind burrowers. Color seems irrelevant to their lives. For the moles, iridescence is likely a side effect of evolution for hairs that allow the moles to move smoothly through, and keep clean in, dirt [64]. Photonic properties may also evolve due to randomness, genetic drift, physical constraint, or evolutionary history rather than to serve a specific biological purpose.
  - In contrast, we propose that the optical structures in heart cockles serve a biological purpose: transmitting necessary sunlight to photosynthetic symbionts. Windows and photosymbiosis are both rare among bivalves, and heart cockles must transmit light to their symbionts in order to survive. The windows transmit significantly more light than typical shells due to specialized optical properties, as our experiments show (Figure 2). The shells' increased transmission cannot simply be attributed to an overall thinner shell; thickness does not correlate with light transmission (Supplemental Figure S7). Therefore, the specific optical properties of the windows seem essential for transmitting light. Further, simulations indicate that the size, shape, and orientation of aragonite fibers sit at a rough evolutionary optimum for transmitting light (Figure 6). Transmission is higher when the aragonite is formed into fibers, when the fibers are co-aligned, and when the fibers are specifically co-aligned along the c-axis or b-axis rather

    than a-axis (Figure 6). These parameter sweeps provide indirect evidence of biologically-selected function.
  - Calculations of real illumination conditions in the tropical oceans also support the idea that shell windows evolved aragonite fibers for a specific purpose: to allow for efficient photosynthesis. *Symbiodinium* photosynthesizes with the highest efficiency around 100 μmol quanta m−2 s−1, and little or no further efficiency is gained from irradiance levels beyond 300 μmol quanta m−2 s−1 [8,65,66]. Consider typical mean irradiance of 410 μmol photons m−2 s−1 at 1 m depth in Singapore, habitat for heart cockles[66]. By adding windows to their shell, heart cockles more than doubled their internal irradiance from 45 to 110 μmol photons m−2 s−1 (i.e., from 11% to 27% of 410 μmol photons m−2 s−1; Figure 2), a range of illumination where those gains significantly improve photosynthetic efficiency. The same calculations apply across heart cockle habitats: irradiance in tropical reefs at depths where heart cockles typically live is on the order of 5 - 500 μmol photons m−2 s−1, depending on depth and shade (e.g., in Okinawa, mean daily maximum irradiance at 3-5 m depth is 401.8 μmol photons m−2 s−1 in exposed reefs and 4.8 μmol photons m−2 s−1 in shaded reefs [67]; in Singaporean fringing reefs irradiance levels increased from 10m depth to 1m depth, ranging 26.3-451.9 μmol photons m−2 s−1[68]; in the North Atlantic Bermuda platform at 8−10 m depth, maximum daily irradiance peaked at 283.90 μmol photons [69]).
- **In addition, we ruled out the possibility that the shells transmit more light merely because they are thinner (see new Supplemental Figure S6)**



- **Supplemental Figure S6. Shell thickness does not correlate with light transmission, suggesting that the optical properties of windows play an essential role in transmitting sunlight for photosynthesis.** We measured shell thickness on the sand-facing and sun-facing side and tested the hypothesis that thinner shells transmit more light. **(A)** The sun-facing sides of shells are significantly thicker than the sand-facing sides of shells (two-sample paired t-test, p < 0.001, mean diff. = 0.3, 95% CI = [0.16, 0.45]). **(B)** For the sand-facing side of shells, shell thickness does not correlate significantly with transmission (two-sample t-test, p = 0.16, cor = 0.46, 95% CI = [-0.2, 0.83]). Indeed, the direction of the non-significant correlation is the opposite of what we expected. **(C)** For the sun-facing side of shells, shell thickness does not correlate with light transmission (two-sample t-test, p = 0.98, cor = -0.01, 95% CI = [-0.61, 0.59].

In addition I also find he paper not recorder and there is a mix-up in the figures: Page 4: I think there's a mistake in the referral.. Figure 4A is, and i quote, "Polished shell fragment shows the fibrousprisms rotating in orientation between the regions 288 labeled 1-3, such that in region 3 we are looking at a cross sections of aligned prisms pointing into the page." it does not suit the text..

- **Thank you for noticing this- our numbering was in error and we have gone through the paper and fixed all the references.**

Page 6: again.... Figure 3B is a schematic of the arrangement.. it is definitely not a "grayscale image encoding height information" and again, they even say that it is (and I quote): "Figure 3: Condensing lenses with truncated caps under the windows focus sunlight. By “truncated”, we refer to the flattened tips of the lens. (A-B) A cross section of a heart cockle shell shows that beneath each shell window in some shells, the aragonite forms a lens shape roughly 750 μm in diameter. The exact size and shape of the lens varies from shell to shell. 226 Beneath the shell is soft tissue with photosynthetic symbionts."

- **We fixed this reference error.**

Page 8: and again... they state that 4B is polished lens SEM image in the figure description.. something went wrong with their numbering…

- **Fixed.**

Reviewer #2 (Remarks to the Author):

The manuscript, "Heart cockle shells transmit sunlight for photosynthesis using bundled fiber optic cables and condensing lenses", is interesting, well-written and deserves to be published in Nature Communications as is. I do have some minor considerations for the authors outlined below.

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In Figure 2 A-D it would be nice to include the illumination images of the sand-facing side of the shells with the same light intesity and image exposure as the portrayed sun-facing sides. This would help achieve the goal of this portion of the figure in giving more of a contextual feel of the system to the reader.

- **This is a great idea. I wish we could do it, but unfortunately we already sliced and polished the shells for our other analyses and can't take additional pictures (since the shells are no longer intact). We will keep this in mind for follow-up work, though, because we agree.**

The references sections of Figure 3 from the main text and the caption to need to be reviewed and updated. Example: line 230 "J-L" there is no "L" in the figure, line 256 "Figure 4 E-G" is probably supposed to be "F-H", etc. Check these all these references for accuracy.

- **We appreciate you catching this; our numbering did indeed go wrong and we have fixed the references in text throughout the manuscript.**

If there are no limitations to figure counts, I would reccommend that Supplemental Figure 3 is moved to the main body of the paper. Since this is a relatively novel system I think that it is necessary to demonstrate that the material is aragonite as opposed to another form of calcium carbonate or other mineral, S3 is the most compelling data to show this.

- **We made this change; supplemental Figure 3 is now Figure 5.**

Overall the paper is highly publishable and interesting. Congratulations on some fine work.

- **Thank you for your feedback and time in reviewing the paper!**

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

The authors have made efforts to improve the manuscript and have added several experiments.
The most important addition is to show no dependence of transmission on thickness.
The biological function is yet missing and all that is presented in this respect is speculation.
Without proving there is a real biological function meas the concept simply dies on the vine.

We appreciate Reviewer 1's previous and present comments, for they strengthened the clarity of our paper. Proving the biological function using behavioral experiments with live specimens is beyond the scope of this paper. Despite that, we do infer the importance of the structures and their impact on biological function. In response to this useful feedback from the reviewer, we have added an analysis of shell thickness as well as textual additions to address the question of biological function.

- See the section entitled "*Determining biological function from optical properties*"
- See also the following additions, regarding function:
    - "We propose that the heart cockle's ability to screen out blue light and UV radiation may be a protective adaptation to resist DNA damage and reduce bleaching risk from high-energy UV radiation. Light stress, and particularly UV radiation, can cause DNA damage, bleaching, and other problems for marine organisms [25–2]"
    - "Shell windows are an adaptation that allow the shallow-living heart cockles to screen solar radiation in a wavelength-dependent manner, due to a combination of at least two physical processes: $CaCO_3$ in bivalve shells has strong absorption in the UV range, and scattering that tends to be inversely proportional to wavelength based on nano-scale inclusions in the shell and the intrinsic birefringence of $CaCO_3$ [30,31]."
    - "Our simulations demonstrate that the truncated lenses condense light with a ~1mm depth of focus beginning around 500-750μm below the bump (Figure 3i-k). We propose that the bumps are an adaptation to help sunlight penetrate more deeply into symbiont-rich tissues[12]."
    - "First, the windows are no thinner than those on the adjacent opaque shell and are, in some cases, thicker. Second, shell thickness does not correlate with light transmission across different individuals (Supplemental Figure S9). Thickness is perhaps the most important parameter in protecting shells against predation[67], and heart cockles seem to have struck a compromise by making the shell a little thinner but getting most of their transmission gains from the optics inside the shell."

Reviewer #3 (Remarks to the Author):

In the paper, “Heart cockle shells transmit sunlight for photosynthesis using bundled fiber optic cables and condensing lenses,” McCoy et al. describes, models and measures the way light passes through the sun-facing shell (in contrast to the sand-facing shell), through a potentially condensing lens and into the algae-rich portion of the animal. The authors expand on the existing theories that the windows allow the bivalve to mechanically protect its soft tissue while letting beneficial light pass through to feed the photosymbionts. As previously shown, the windows are made from the same materials as the shell only organized/crystallized into bundles of elongated spires. The authors expand on this knowledge by exploring through microscopy, how the cockle shell’s windows project images and by modeling how differently sized fibers result in more or less transmission. The authors also find that some of the windows have bumps on the inside of the shell and explore the existing idea that these may serve as lenses and find they have focal distances along the order of where it has been shown that most of the photosymbionts are located.

While this work mostly expands on existing observations and theories, the experiments are creative and informative and models are new. This paper however, touches the surface of many of these ideas without going into the details I would like to see and leaves me wondering more rather than understanding comprehensively how the system is working or how evolution led to this point (or both). This aspect could be improved by either focusing in on a particular optical piece of the system or by viewing the system as a whole and showing all the interactions and how they lead to the most desired result. The following are more specific comments that could help in either direction.

Thank you for your very useful comments. We reply in detail below, and the paper is much stronger as a result of the new additions!

- I don’t fully grasp the idea that these are the first “fiber optic cable bundles” .This is stated in the abstract and in the results section. What constitutes a bundle? I understand that there are few to many instances of fiber optic cables. Are the bundles simply that they are organized into smallish shapes - the windows - and not consistent throughout? Or, is actually that within the windows, there are separate bundles of fibers? And, why would they need/use these fiber optics fibers in the form of bundles if they are within the window?

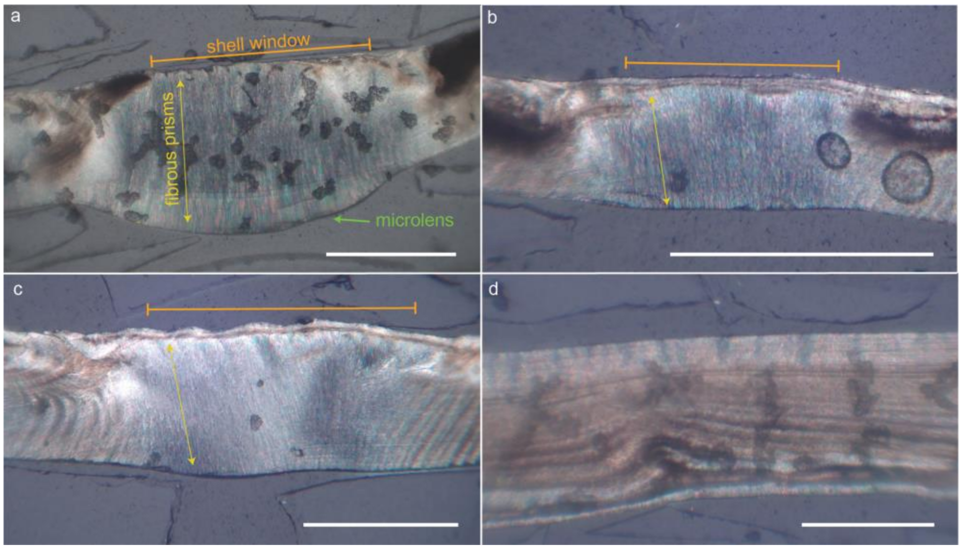
A bundle is composed of aligned fibers in a bound array. In fact, fiber bundles that are aligned can transmit images and (in engineering) are termed “flexible image carriers” (Hecht, Optics, 5th Edition p 206-207). Rigid bundles (non flexible) are called “mosaics,” and the mineral ulexite (mentioned in our study) is a natural mosaic. In these shells, each window is itself a fiber optic cable bundle that can transmit images. That is, the windows are composed of many co-aligned fibers. These are helpful clarifying questions, and we added the following text:

> “Fiber optic cable bundles (also termed “mosaics” in their rigid form) are bound, co-aligned fibers that can – if the fibers are co-terminal– transmit images (Hecht 2017). Each shell window is itself a fiber optic cable bundle.”

- The above leads me to another comment: More information on average sizes of the windows, fibers and bundles is needed as well as their extent in the z direction along with variance would be informative. This information is hard to glean from any of the pictures or microscope images. I also think a more detailed image or drawing with increased magnification of the windows , fibers and bundles would help one “visualize” the system - like Figure 6b.

We prepared polished thin sections of seven shells in order to measure the extent of the fibers in the Z-direction and better visualize the system. We added supplemental figure S6, as well as the following measurements and textual additions:

- “When viewed from above, triangular shell windows average 0.71 mm^2 in area (n=50 windows, Figure 2a) and stripes averaged 0.58 mm in width (n=14, Figure 2b).”
- “The aragonite fiber optic cable bundles extend through an average of 83% of the shell thickness (range= [70%,99%]; n=9 polished cross sections of windows; see Supplemental Figure S6); the fibers either terminate at a crossed lamellar portion of aragonite or the outer protective periostracum layer of the shell.”



Supplemental Figure S6: Fibrous prisms of aragonite comprise, on average, 83% of a shell’s cross-sectional thickness. Orange bar indicates window region of shell, and yellow arrows indicate the direction of the co-oriented aragonite fiber optic bundles. These photographs were taken with a birefringent microscope, showing polished cross-sections of **(a)** shell window with microlens (green text), **(b)** polished shell window, **(c)** polished shell window, and **(d)** polished cross-section of sand-facing side of shell with no windows. Dark spots are pigmentary and other inclusions in the shells. All scale bars are 0.5 mm.

- In the Results section on the transparent windows, the authors state they cut flat 1 cm^2 fragments. What does flat mean in this context? Meaning there isn’t must variation in shell thickness at 1 cm^2 areas? What is the typical shell thickness where the windows are and are the windows the same thickness as the shell aside from the lenses?

Good question– there is some natural curvature and we meant to say that we selected a relatively non-curving section of shell. We changed the text to read “Using a lapidary saw, we cut nearly-flat 1 cm2 fragments from heart cockle shell specimens (i.e., a section of shell with minimal natural curvature).” The windows are indeed the same thickness as the opaque regions of the sun-facing side of the shell, unless they have a lens.

- in relation to the above, in the supplemental information, the authors show how shell thickness does not correlate with transmission which is hard to wrap my head around. Is this because the measurements are taken with an integrating sphere so only absorption would cause a decrease in transmission, where there isn't much? Also, the polished shells at 300 microns thick do show a significantly increased transmittance. Would that be because they are polished? If one were measuring only transmittance (without an integrating sphere), I would expect that scattering (and absorption) would effect how much light is transmitted through, therefore a thicker, scattering material would result in less transmittance than a thinner one. Clarity on this should be in the manuscript especially since the sun-facing shell is overall thinner than the sand-facing shell and that thickness and optical thickness in optics is an important and informative parameter. I bet someone has measured the scattering and absorption coefficients of bivalve shells in general and the authors should cite this and understand it.

We are glad you pointed this out, since this was a lack of clarity in our writing. As you say, the thickness of any optical material has a strong effect on transmission! And the sun-facing side of shells is significantly thinner than the sand-facing side, which *does* increase light transmission. But we showed that thickness cannot *alone* predict transmission. We edited the text to read: **“The sun-facing side of shells is significantly thinner than the sand-facing side (Supplemental Figure S7), but increased transmission cannot simply be attributed to an overall thinner shell for two reasons. First, the windows are no thinner than those on the adjacent opaque shell and are, in some cases, thicker. Second, shell thickness does not correlate with light transmission across different individuals (Supplemental Figure S7). Thickness is perhaps the most important parameter in protecting shells against predation67, and heart cockles seem to have struck a compromise by making the shell a little thinner but getting most of their transmission gains from the optics inside the shell.”**

We measured many different individuals to test whether the thickness of the shell across individuals was a significant factor in driving transmission; it was not. We clarified the text as follows: **“Across different individuals, shell thickness does not correlate with light transmission, suggesting that the optical properties of windows play an essential role in transmitting sunlight for photosynthesis. We measured shell thickness on the sand-facing and sun-facing side of 11 shells and tested the hypothesis that thinner- shelled individuals transmit more light.”**

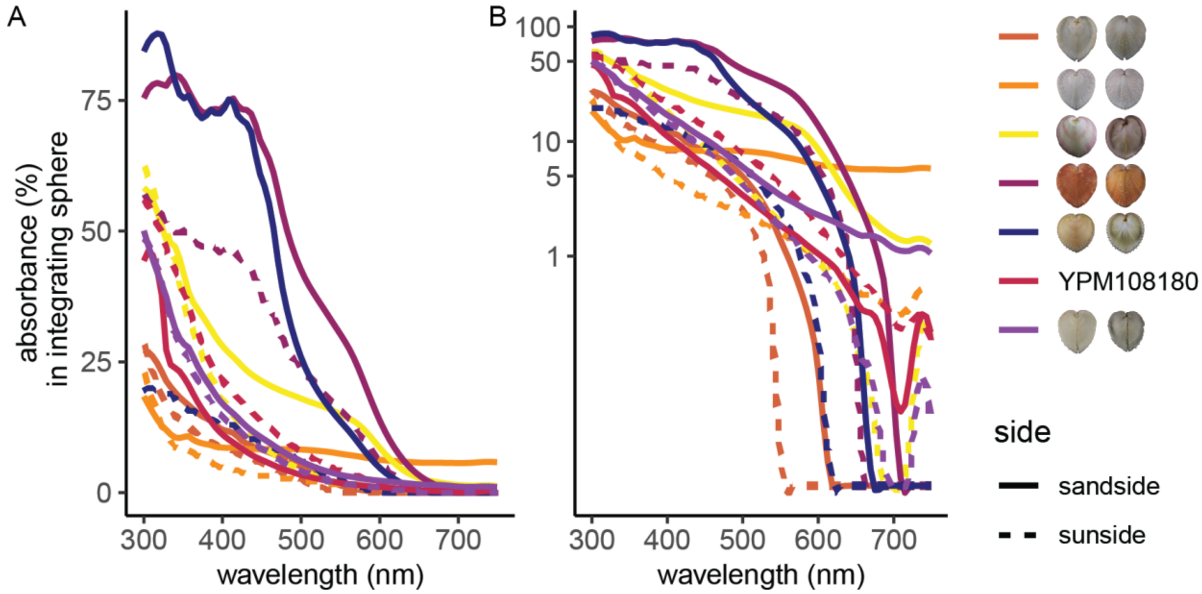
- Also, what wavelength is Supplemental figure s7 measured at? If measured in the UV, would you see a difference?
This measurement does include UV; it is across all wavelengths from 300 nm to 700 nm (as in Supplemental Figure S2), which we should have made clear; we added that information to the caption. “We measured transmission across 300nm - 700nm.” The general shapes of the curves (lower transmission in UV) can be seen in Supp Fig S2.

- Can the authors cite the reference to absorption and scattering of Calcium Carbonate? And find a reference that shows absorption and scattering of similarly composed shells.
We added the following: “$CaCO_3$ has strong absorption in the UV range(Sulimai et al. 2019; Li and Ortiz 2013),” and at your suggestion directly measured the absorbance in the shells (see below). The fiber-optic aragonite prisms in these shells are not otherwise known in nature.

- Also, what physical/optical aspect about the windowed side allows statistically more visible light than UV light when compared to the sand-facing side? The mention of Calcium carbonate is true for both sides of the shell. (Though I can’t find where shells scatter light like 1/lambda - can the authors cite that?) This is again a section where shell thickness is a natural first guess and perhaps scattering and absorption is non-linear with wavelength resulting in nonlinear changes in transmittance. But, if it is the windows and how they are designed, that would be interesting and relevant and the authors should show/describe it.
We added a new figure showing absorbance through the sun-facing and sand-facing sides of the shell (Supplemental Figure S6), at your highly useful suggestion, and now the relevant text reads:

- “Further, we measured absorbance by placing seven shell fragments in seawater inside an integrating sphere. Shells absorbed light in a nonlinear, wavelength-specific manner (Supplemental Figure S3). The sand-facing side absorbed a median 1.5% of 600-700nm red light (range = 0-6%), compared to 31% of 300-400nm UV radiation (range = 12-80%). The sun-facing side absorbs 0.1% of 600-700nm red light (range = 0-2%), compared to 30% of 300-400nm UV radiation (range = 10-51%). Across all maesured wavelengths, 300-700nm, the sun-facing side absorbed less light than the sand-facing side (sun-facing: median=10.3%, range= (4%-26%); sand-facing side: median 11.8%, range=(7.2% - 43.5%)). Pigmented shells seem to absorb more (see Supplemental Figure S3).”
- “Shell windows are an adaptation that allow the shallow-living heart cockles to screen solar radiation in a wavelength-dependent manner, due to a combination of at least two physical processes: $CaCO_3$ has strong absorption in the UV range (Sulimai et al. 2019; Li and Ortiz 2013), and scattering tends to be inversely proportional to wavelength.”

The new figure is:



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- **Supplemental Figure S3: Heart cockle shells absorb light in a wavelength-specific manner.** We measured the absorbance of seven shells. The same data are plotted here on **(A)** a normal scale and **(B)** a log scale. Shells with more orange/yellow pigment seemed to have greater wavelength-specific absorption (dark blue and purple lines). For these measurements, a flat rectangular piece of shell approximately 1.5 cm by 1 cm was immersed in seawater in a cuvette. The cuvette was placed inside an integrating sphere and illuminated with normally-incident light. For specimen YPM 108180, no photograph was available for legend inset.

- In Figure 2, the grey and black lines are mislabeled in the caption.
Good catch- fixed.

- in the Condensing lenses section, the authors mention they found some individuals with lenses. Why only some individuals? Does that mean this aspect is not as important? And, were the lenses found on all the windows or only in a specific portion of the shell?
The short answer, we think, is that individual variation is everywhere in biology. The lenses were only found beneath the windows, not elsewhere. And it may be true that since only some individuals have lenses, that aspect is not as important for light transmission. We added some text to the discussion:

- "...located exclusively beneath each window"
- " Only some heart cockle individuals have lenses, suggesting that there is more to the evolutionary story than we currently know."

- The authors show that the lenses/bumps focus light to around 1 mm with modeling and experiment. They discuss how this may be to reach the symbionts but in order to solidify this argument, more evidence beyond what is cited would be helpful. Is there any way for the authors themselves to experimentally show the location of symbionts and how the rest of the tissue is organized the lenses?

This is a fantastic idea, but unfortunately we have not yet found live heart cockles (despite looking for many hours on our latest fieldwork trip to Palau). We will have to prioritize this question for future research.

- question from a non-expert: Are the micro lenses and windows evolved from eyes?
Great question! Certainly the image projection plus focusing make this a tempting theory. Once we find some live heart cockles, we will do some testing in the lab.

- in the lens simulation, it would be nice to have a depth in addition to a scale bar. Also, why doesn't the simulation extend beyond the depth shown? It would also be interesting to know where the focal point ends.
Supplemental Figure S5 shows simulation results extending beyond the depth shown, as you suggested– we have added a reference to Supp Figure S5 to the caption of the lens simulation figure. It simply made the main-text figure rather too large to include the full depth in the main manuscript figure.

- "lensing for other wavelengths of light, and with an extended vertical-axis, is shown in Supplemental Figure S5"

- It's neat that the optical fiber bundles project images, but what would the significance of this be biologically? And/or can you use this observation to make quantitative or characteristic determinations about the underlying structure? Showing this is fine and neat but making it more relevant to the biology or to measurement is necessary.
This observation shows us that the underlying mineral is indeed acting like a fiber optic cable bundle. As you say, the biological significance is not so clear! We added this text:
"It is not clear that projecting images is of any use to the heart cockle; perhaps the lenses are associated with sensory perception rather than only photosynthesis. However, these experimental results show that the mineral is acting as a bundle of fiber optic cables."

- The paragraph on Anderson localization is also interesting but mostly descriptive and the authors provide no evidence that this system transmits light by that effect.
We deleted this paragraph.

- The authors make a statement that since the transmission spectra do not show noticeable absorption peaks, the UV radiation must be screened primarily by imperfections in the fiber optic structures. While it's true that there are no peaks (except for the colorful shells) the significant drop in UV for both shells indicates absorption (and scattering) especially since this measurement is with an integrating sphere. Unless there is significantly more UV reflection or back scattering - which the authors would have to show to make this claim.
- If the authors put the shell inside an integrating sphere, they could measure exactly the absorption.
Good idea! We measured absorbance as you suggested, which was a great idea and strengthened our paper. We added a new Supplemental Figure S6, copied above in our response to reviewers along with the corresponding changes to the text, and also changed the text you refer to in this comment as follows:

- “In heart cockles, the windows seem to screen UV radiation through two mechanisms: first, scattering due to imperfections in the fiber optic structures, and second, wavelength-specific absorption from $CaCO_3$ and yellow/orange shell pigments (Supplemental Figure S6).”

- The authors state that 1 micron diameter fibers is superior to small fiber diameters in the visible and only slightly inferior. By looking at their plot, I would argue that larger diameters result in significantly higher transmittance. Would there be a mechanistic reasoning why the bivalve would not want even higher transmission?

We made the following edit:

- “The ~1μm diameter of the observed fibers is superior to smaller fiber diameters for light transmission, but inferior to all larger fiber diameters (Figure 6d). We speculate the aragonite fibers evolved to that width as a compromise between light transmission and mechanical toughness.”

Generally, the fibrous prisms of aragonite are not the arrangement that gives a shell maximum hardness and resistance to predation. The crossed lamellar morphology of aragonite is widespread in shells and lends stronger architecture, therefore protecting the shell against predation. So we suspect that the mechanistic limitation on even higher transmission is balancing multiple selective pressures (predation / toughness versus transmission). The same reasoning might explain why the shell has windows rather than being 100% clear.

REVIEWERS' COMMENTS

Reviewer #3 (Remarks to the Author):

The authors have addressed most or all of my concerns and as a result, I think their paper is more comprehensive and informative.

One mistake that I noticed is that in figure S9, part (a) states: “The sun-facing sides of shells are significantly thicker than the sand-facing sides of shells”. I believe it should be: “The sun-facing sides of shells are significantly thinner than the sand-facing sides of shells.”

- **We fixed this– good catch.**

On line 165, you mention screening blue light and UV radiation but there is no mention of blue light for the rest of the paper. My first thought when reading that is that blue light is between 400-500 nm and is spectrally where algae absorb the most light so why would they want to screen “blue” light. It’s nuanced but my advice is to just stick to UV in verbiage.

- **We also fixed this.**

Otherwise, great job improving the paper’s impact.

Reviewer #3 (Remarks on code availability):

I did not install and run the main code but it is in R which is readily used by many. The code looks good. There is no readme file but there are lots of comments that would help someone understand what's going on in the code. The rest of the files are easily accessible and in readable formats. The FDTD simulations are created/solved in Lumiercal, a commercially available software that I don't have but should be reproducible given all the parameters.

- **Thank you for your useful comments here and previously!**