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July 27th, 2018

**RE: Revised article submission Jin & Riedel-Kruse “High-resolution patterned biofilm deposition using pDawn-Ag43”**

Dear Editor,

Thank you for your time in assessing our original submission and coordinating with reviewers. We are pleased to submit our revised manuscript, “High-resolution patterned biofilm deposition using pDawn-Ag43” for consideration for publication in *JOVE*. All authors have approved this manuscript.

We would like to thank the reviewers and editors for their time and thoughtful comments. Please find below a copy of the original comments, alongside our responses (italicized) detailing the corresponding changes in the manuscript. We carried out the suggested changes, and we believe we were able to address all the comments in full.

Editorial comments:

Changes to be made by the Author(s) regarding the written manuscript:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.
   1. *We have gone through the manuscript again multiple times to ensure there are no spelling or grammar issues.*
2. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. “This figure has been modified from [citation].”
   1. *Please find the permission information in the ‘reprintPermission.docx’ file. We have cited where appropriate in the figure legend.*
3. Figures: Please include a space between all numbers and their corresponding units, i.e., -80 °C (Figure 1), 460 nm (Figure 2), 50 µW/cm2 (Figure 2), 8 mm (Figure 4), 100 µm (Figure 4), etc.
   1. *Thank you for pointing this out, we have added spaces where appropriate*
4. Please shorten the figure legends. The Discussion of the Figures should be placed in the Representative Results. Details of the methodology should not be in the Figure Legends, but rather the Protocol.
   1. *Thank you for this suggestion, we have shortened the figure legends as requested and moved the bulk of the text to the Protocol section.*
5. Please use SI abbreviations for all units: L, mL, µL, h, min, s, etc.
   1. *Thank you for pointing this out, we have corrected where appropriate*
6. Please include a space between all numbers and their corresponding units: 15 mL, 37 °C, 60 s; etc.
   1. *Thank you for pointing this out, we have corrected where appropriate*
7. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. For example: Addgene, Microsoft Powerpoint, etc.
   1. *We have removed commercial language as appropriate and moved them to the Table of Materials and Reagents*
8. Please revise the protocol to contain only action items that direct the reader to do something (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” Please include all safety procedures and use of hoods, etc. Please move the discussion about the protocol to the Discussion.
   1. *We have made changes throughout the protocol section to ensure imperative tense whenever possible, and added “Note” as appropriate, while also moving several points to the discussion section.*
9. Please add more details to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.
   1. *We have increased the level of detail in the protocols throughout, as well as specifically in addressing the following three points*
10. 1.1.4: Please specify the temperature for growing the bacteria.
    1. *Thank you for pointing this out, temperature at 37 deg C is now explicitly stated*
11. 1.2.2: Please specify at what wavelength the OD is recorded.
    1. *Thank you for pointing this out, OD600 is now explicitly stated*
12. 4.6: Please describe how this is done.
    1. *Thank you for pointing this out, given the multitude of variations on confocal microscopy for biofilms, we have added a reference on confocal microscopy for reference.*
13. After you have made all the recommended changes to your protocol (listed above), please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.

14. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.

15. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.

* 1. *Video section has been highlighted*

16. Please reference all data and figures in the manuscript. Currently Figure 3 and Table 1 are not described in the manuscript.

1. *Thank you for pointing this out, we have modified the text to more closely refer to the figures, and Table 1 + Figure 3 are now explicitly referred to.*

17. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:

a) Critical steps within the protocol

b) Any modifications and troubleshooting of the technique

c) Any limitations of the technique

d) The significance with respect to existing methods

e) Any future applications of the technique

1. *We have added a large section on critical steps of protocol, and reference modifications and troubleshooting in Table 1. We have also expanded section on technique limitations and existing methods to address comments by Reviewers 1 and 2.*

Reviewers' comments:

Reviewer #1:

In the manuscript, the authors provided a well-written protocol based on their previously published paper ("Biofilm Lithography enables high-resolution cell patterning via optogenetic adhesin expression." PNAS 115(14): 3698-3703), which allows readers to follow their method easily and reproduce the patterned biofilms formed by E. coli. In this manner, I suggested that the manuscript should be published. Before that, there are some technical issues that have been to be addressed.

1) Tuning the bacteria-surface interaction is the key to control the formation of patterned biofilms. Here, the authors regulated/controlled the expression of an adhesion protein on the E. coli via optogenetics tools. But this strategy may not work well in the case that bacteria-surface interaction is sufficient strong, for example, i) E. coil with a surface modified by polylysine; ii) bacterial species, like Pseudomonas aeruginosa or Bacillus subtilis, can spontaneously attach to the surface. This point should be explicitly mentioned in the section of discussion, which can help the readers to understand the limitations for applying this method in different scenarios, including different surfaces and/or different bacterial species.

*Thank you for pointing this out, we have updated our discussion section to mention this potential limitation*

2) A key reference published in the ACS synthetic biology should be cited (Huang, Y. J., et al. (2018). "Bioprinting Living Biofilms through Optogenetic Manipulation." Acs Synthetic Biology 7(5): 1195-1200) , where Huang et. al. provided a different strategy to show that patterned biofilms of Pseudomonas aeruginosa can be printed on surfaces.

*Thank you for pointing this out, we included this in our updated discussion section*

3) L. 62 e coli -> E. coli

*Thank you for pointing this out, we have now corrected this typo.*

Reviewer #2:

Manuscript Summary:

The paper of Jin & Riedel-Kruse describes a protocol for patterning of E.coli biofilm using blue light activated expression of the adhesion protein Ag43. It is complementary to their PNAS 2018 paper describing this system. There are a couple of minor issues that need to be addressed.

Minor Concerns:

(i) Two essential references are missing: one on the paper describing the pDawn plasmid (Moeglich's lab), another on of the first paper describing bacterial photolithograthy (Voigt's lab).

*Thank you for pointing this out, we have now added these references as well as expanded the discussion on previous work.*

(ii) p75: Describing E. coli at OD 0.4-0.8 as a "late exponential phase" is inaccurate. Delete 'late'.

*Thank you for pointing this out, we have now corrected this mistake.*

(iii) It would be helpful to have some guidance about the choice of a projector. How does one reproduce the protocol if the projector model listed in the manuscript is no longer available? Same guidance would be useful for the choice of an optical power meter.

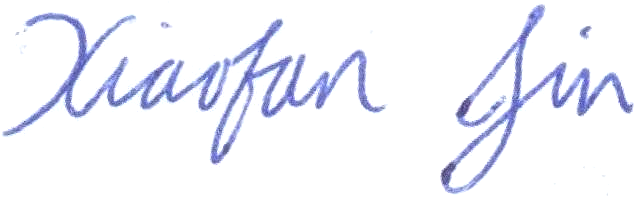
*Thank you for pointing this out, we have updated the JOVE Table of Materials to provide guidance on projector and power meter choice, pointing out that the exact models listed are not the only ones that will work.*

(iv) Is there a reason to place the projector at the bottom and tape a plate at the top? It seems counterintuitive. Explain.

*Thank you for pointing this out, we have updated the discussion section to mention this point: “Note in this protocol we describe an inverted illumination setup where the projector shines light from below upwards towards the biofilm sample. The advantage of this setup is that the light only needs to travel through the bottom of the culture dish before reaching the biofilm formation surface. Illuminating from above means that the light would have to travel through the liquid media above the biofilm surface, which during the course of growth gets cloudy with planktonic cells.”*

We look forward to hearing back from you regarding this revised submission, and are happy to respond to any further questions and comments.

Sincerely,



Xiaofan Jin Ingmar Riedel-Kruse