Reply to Editor

Thank you for the valuable suggestions and constructive comments regarding our manuscript. We have revised the manuscript in accordance with your suggestions and comments. We have included our point-by-point responses to your comments below in red. In addition, changes in the revised manuscript in response to the comments are written in red.

Editorial comments:

Changes to be made by the author(s) regarding the written manuscript:
Editorial comments:

1) Please see the attached word document. In-text comments have been made; these require your attention. Please address the comments by editing your manuscript/figures. Please maintain the current format and track all your edits.

Line 100: PROTOCOL
Some steps were edited for language and structure to meet JoVE's requirements.

Thank you for editing.

Line 107: N2[10]
Add all worm strains to the materials table.

We have added three worm strains to the material table.

Line 114: NGM
Add to the table of materials

The nematode growth medium (NGM) is not a product. As referred in this sentence, the NGM is prepared based on the previous established method (ref #10). The "NGM" is the well-known term for worm researchers, we think that the additional information is not required.

Line 114: *E. coli*
Add the strain to the table of materials

We have a bacterial strain to the material table.

Line 159: platina picker
Add to the table of materials.

The platina picker has been already included in the material table.

Line 191: pick up

Is this done under a microscope?

Yes, this is done under a microscope. We have added this information.

Line 215: ≤3 µL
The range is a bit open ended. Can you provide an upper limit?

The droplet is approximately 2-3 µL. We have revised the description.

Line 229: microscope
Mention magnification

As an example of the possible microscopes using for this purpose, we have already included a stereomicroscope in the material table.

Line 232: a dedicated sheet
Spreadsheet? Sheet of paper? Since you talk about worm sheets, this is a bit confusing.

This is a sheet of paper. In accordance with your comments, we have changed the term “sheet” to “sheet of paper”.

Line 232: (Supplement 1)
Not provided with submission files.

We are so sorry. We have provided the file.

Line 248: ≥10 µL
The range is a bit open ended. Can you provide an upper limit?

The droplet is approximately 10−15 µL. We have revised the description.
In concern with this, we have newly selected this section (Protocol 5) for filming.

Line 248: observe
Is a microscope needed? If so mention the magnification.

Yes, this is done under a microscope. We have revised the description.
As an example of the possible microscopes using for this purpose, we have already included a stereomicroscope in the material table.

Line 254: irradiation
Please reference the step number.

We have revised to refer to Protocol 7.9.

Line 254: immobilization
Please reference the step number.

We have revised to refer to Protocol 4.2−4.5.
In concern with this, we have moved Protocol 4.3 to before Protocol 4.1.

Line 268: 6.1. Select a ⋯
Nothing to film so I have unhighlighted.

We agree with your suggested changes.

Line 268: fluorescence microscope
Please provide additional specifications, e.g. excitation light source intensity and wavelength. Emission filter wavelength, lens magnification and N.A.

The specification of the fluorescence microscope system used for in Protocol 3 is as follows:
Excitation filter wavelength: 425-445 nm
Emission filter wavelength: 460-510 nm
Lens magnification: x2
N.A.: 0.3
However, the needed specification of the system depends on both users and/or the purpose of observations, and our system shown in this paper is only an example of possible systems. Therefore, we did not describe the specification in the main text and only referred to the material table.
We have updated the material table and have added the description in Note of Protocol 6.1 as follows:
The specification of the fluorescence microscope system used for in this paper is shown in **Table of Materials**. However, the needed specification is not limited to our example because it depends on the purpose of observation or/and users.

Line 269: (Figure 3A)
Add the microscope, optical components and camera to the table of materials.

We have updated the material table.

Line 271: objective lens
Magnification and N.A?
As described above, the lens magnification is x2, and N.A. is 0.3.
However, the needed specification of the system depends on users and/or the purpose of observations, and our system shown in this paper is only an example of possible systems. Therefore, we did not describe the specification in the main text and only referred to the material table.

Line 274: section 1
I added this for clarity. Please verify that this is okay. I have unhighlighted this as it lacks filmable content.

Thank you for this revision. We agree with this revision.

Line 277: adult HBR4[11]
Please add the strains to the table of materials.

We have added the strain to the material table.

Line 287: microscope
Please provide additional specifications, e.g. excitation light source intensity and wavelength. Emission filter wavelength, lens magnification and N.A.

As described above, we have updated the material table and have referred to it.

Line 289: established methods[14–16]
I made this a note and unhighlighted it. Anything you wish to film must be described in the current manuscript.

We agree with your revision. There is no additional information to be filmed.

Line 290: ⋯ present study are in general use.
Unclear what is meant.

We have revised the description as follows:
Follow the previously established methods[14–16], since the microscope observation methods (including fluorescence observation) and the specification of the microscope system depend on the purpose of observation.

Line 292: Image
This step lacks details. What is the dye concentration?

We have no evidence on the dye concentration and don't intend to add descriptions.

Line 292: calcium-ion
A calcium dye was not used so far. Please add a step to describe this.
We have no evidence on the dye concentration and don't intend to add descriptions.

Line 300: microbeam
What kind of radiation beam is used? Gamma-photons?

We used heavy ions such as carbon ions.
We have added the information.

Line 405: (~30 mL/(24 h·m²·MPa)
Reference? Please double check that the units are correct

It is well-known that PET has low oxygen transmission, which is about 100 times lower than that of PS. However, the value depends on the product (thickness, fabrication method, etc.).
We have removed the value from this sentence, and instead of this, we have referred to the catalog (ref. new #17) regarding to PET film (TORAY Lumirror) used in the Protocol 3. The data shown in the catalog indicates the oxygen transmission rate of PET films is about 100 times lower than that of PS films.

Line 406: 100 times lower than that of PS
Reference?

As described above, we have referred to the catalog of PET film (ref. new #17) in this sentence.

Line 422: microbeam irradiation
Are any results available to demonstrate the outcomes of the irradiation? This can be cited as a reference to a previous publication.

We are sorry, but we have no data to be included in this paper.
Actually, we have submitted another paper regarding this microbeam irradiation of *C. elegans*, and we may refer to it here if it was published before this paper.

Line 463: each group. Error bars represent standard error of the mean of five independent experiments. All data were analyzed using one-way ANOVA at the 0.05 (*) or 0.01 (**) significance level. From the data no significant differences were observed? Correct?

We forgot to indicate asterisks (* and **) on the graph.
We have revised the graph and provided the revised Figure 5.

Line 472: Supplement 1
File missing

We are so sorry. We have provided the file.

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Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

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