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Letter to the Editor

Toxicity of Visible Light Follows a Rule Similar to that for X-Ray Damage

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The paper entitled "Less is More: Longer Exposure Times with Low Light Intensity is Less Photo-Toxic" published in the November 2017 issue of *Microscopy Today* [1] reports an interesting study regarding the relationships between the light intensity and its biological effects. The authors of this paper suggested that one can eliminate the photo-toxicity by lowering "temporal light dose." We would like to propose an alternative parameter that can better describe the results: light power.

In the abovementioned paper, the "temporal light dose" (mW/s) seems to be calculated by dividing the light power (mW) by the exposure time (s). However, the light power (mW) itself is defined as the rate of energy transfer per second (mJ/s) and is already divided by time. The dimension of the "temporal light dose" represents time variation of the light power, although no information regarding the time variation of the light power is presented in the paper.

A plausible parameter "light dose" (mJ) can be calculated by multiplying the light power (mW) by the exposure time (s). For the conditions shown in Table 1 of the abovementioned paper, the light dose is 0.57, 0.47, 0.42, or 0.39 mJ for the experiment using the light power of 1.64, 6.68, 12.03, or 22.10 mW, respectively. Differences between these light doses are small. This is already stated by the authors that "the total light exposure to the sample was similar for each image". Therefore, the light power rather than the light dose should be the essential factor of the photo-toxicity.

Figure 1 of the abovementioned paper [1] shows a pseudo-linear relationship between the photo-bleaching decay rate and the "temporal light dose." However, the decay rate shows an exponential correlation by plotting it against the light power (Figure 1 (revised)).

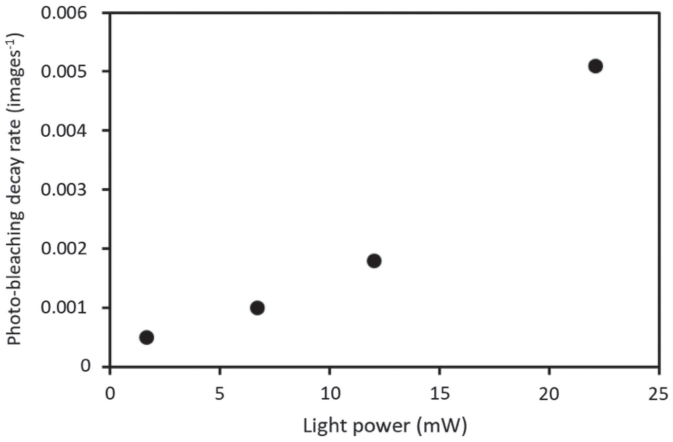

Figure 1 (revised): Photo-bleaching rates for fixed cells.

This is consistent with the exponential model of the biological effect of X-ray or UV irradiation [2], suggesting that the toxicity of visible light follows a rule similar to that of the X-ray damage. Thus, Figures 2, 3, 7, and 8, as well as the text and Table 1 [1], should be reinterpreted by taking this into account. The results indicate that the photo-toxicity can be eliminated in an exponential manner by lowering the light power. This reinterpretation will not impair the paper contents, but rather it strengthens the findings originally reported in the abovementioned paper [1].

References

[1] F Mubaid and CM Brown, *Microscopy Today* 25(6) (2017) 26–33.
[2] L Bodgi et al., *J Theor Biol* 394 (2016) 93–101.

Authors' Response

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In their Letter to the Editor statement above, Saiga and Mizutani make some insightful comments regarding the parameters used in our paper "Less is More: Longer Exposure Times with Low Light Intensity is Less Photo-Toxic." We thank the authors for taking the time and bringing these issues to our attention. The authors are correct that the major factor affecting photo-bleaching

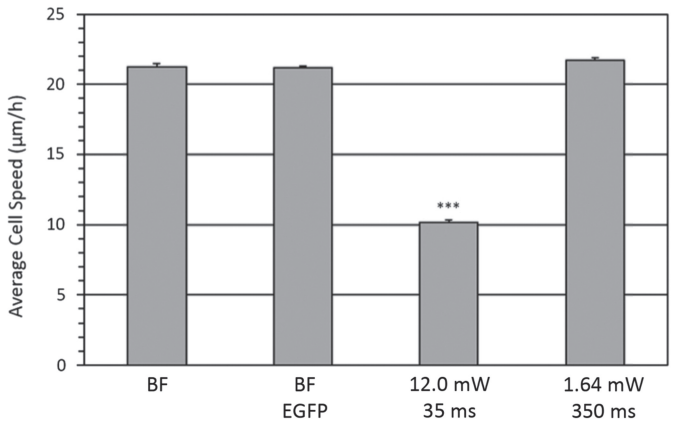

Figure 5 (revised): Average cell migration speeds compared to bright-field controls.

and, in turn, photo-toxicity is the power of the light. In the paper, the camera exposure time was adjusted so that the light dose would be similar for each image with different incident light powers. In writing the article, we chose the "temporal light dose" because it is very straightforward and accessible to a wide array of microscopy users. Microscopists can easily measure incident light power with a 10× lens as was done in the paper and then divide that number by the exposure time to calculate the "temporal light dose." As seen in Table 1 of the paper, the smaller the temporal light dose value is, the better is the imaging condition. After reexamining this calculation following the Letter to the Editor above, we realized that if power is left constant and the exposure time is increased then the temporal light dose would decrease. However, a longer exposure time would not result in reduced photo-toxicity and would not improve cellular health. Thus, instead of the "temporal light dose" parameter we would like to recommend that researchers keep the light power at a minimum and increase exposure time as much as required to generate a good signal-to-noise image. However, this may not always be possible as shorter exposure times may be required to image rapid biological processes. In this case, keeping the light power at a minimum is still essential.

An interesting observation made in the above Letter is the exponential correlation between the decay rate of fluorescence and the power. This is in line with the two-step photolysis

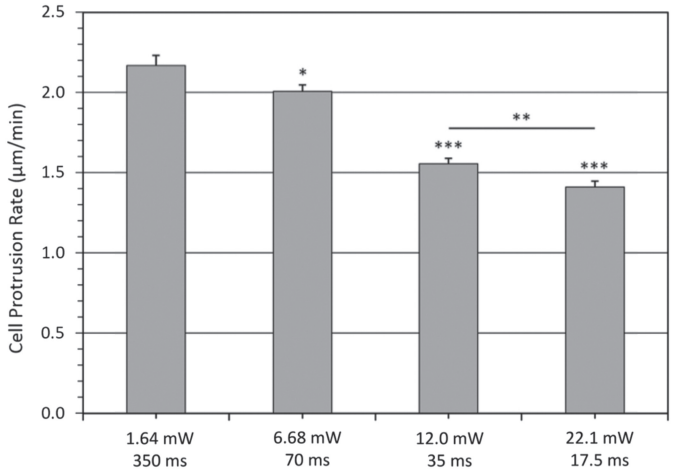

Figure 7 (revised): Average cell protrusion speeds versus increasing light power.

mechanism of photo-bleaching, which strongly depends on light power intensity that has been previously published [1]. This is consistent with our data and the thought that the photo-bleaching and photo-toxicity are mainly linked to photo-lysis of triplet state fluorescent molecules. In fact, if the data were collected so that the camera exposure time was kept constant and only the incident light power was changed, the exponential trend of the photo-bleaching rate with increasing power would be even more prominent.

The authors of the Letter also asked about the temporal variation of the incident light intensity. We have in fact measured that for many LED-based light sources, and it is well below 0.5% on all time scales we measured (ms, seconds, hours, days).

We have reproduced key figures from the article, related to live cell imaging, in terms of power rather than temporal light dose. Both cell migration speeds (Figure 5 (revised)) and cell protrusion speeds (Figure 7 (revised)) are significantly reduced at light power of 12 mW compared to 1.64 mW. In our original article conditions of high light dose and low light dose were 12 mW for 35 ms and 1.64 mW for 350 ms, respectively.

In conclusion, we would like to thank the authors of the Letter to the Editor for helping us clarify our parameters. We no longer recommend using the "temporal light dose" parameter but minimal light power and longer exposure times to minimize photo-toxicity.

References

[1] LA Deschenes and DA Vanden Bout, *Chem Phys Lett* 365(5–6) (2002) 387–95.

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