Laser Without Dose? Problems to Define the Applied Dose in Laser Micro Irradiation Experiments

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The rise of laser micro irradiation (LMI) as a new tool to generate DNA double-strand breaks (DSBs) started in the late 90s, when this kind of systems showed the capability to irradiate specified sub-nuclear regions and to simultaneously observe fast and early damage responses. Although, a comparable experimental performance can also be realized with heavy ion (HI) irradiation, in comparison to LMI systems HI experiments have to face up their disadvantage of a much more complex setup. However - despite all advantages - one of the major drawbacks of LMI systems is the lack of any reliable measurement of the applied dose. The most promising approach to overcome this problem was described by Bekker-Jensen et al. (2006) who compared the amount of irradiation induced Replication Protein A (RPA) foci after LMI ($\lambda = 337$nm) and X-rays as a reference irradiation. The resulting dose effect curve for X-rays allows the allocation of the LMI data point at a nominal dose of 3Gy. Our results show that this holds true only if the foci are counted within the whole nucleus but varies strongly when the analyzed area (ROI) is reduced. While the homogenous dose distribution of X-rays results in a linear scaling of the foci number with respect to the area of the ROI, the number of RPA foci induced by LMI does not scale with the area of the ROI until the ROI is smaller than the irradiated region. The problem of a comparison of radiations with homogeneous and inhomogeneous dose distribution is also reflected when the method of Bekker-Jensen et al. (2006) is applied to HI irradiation. In this case even the dose of high LET Xenon ions (28Gy) is assigned to a nominal dose of about 2Gy. In conclusion, we were able to show that by shrinkage of the ROI the bias of inhomogeneities in the dose distribution of LMI and HI irradiation can be reduced and that the resulting dose effect curves of X-rays and HIs can be combined. Unfortunately, the laser data point is located in the saturation region of this dose effect curve (0.7 foci/μm^2) making a unique allocation of the laser dose difficult. However, using this method, dose equivalents of locally applied laser irradiation is most probably in the order of a few hundred Gray within the irradiated volume.

Bekker-Jensen et al. (2006), JCB 173(2): 195-206

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