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Improved Imaging of a Biological Model for a Preclinical Proton Minibeam Radiotherapy Facility

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In collaboration with the Helmholtz Zentrum Berlin (HZB) a new pMBT facility, called MINIBEE, is currently under construction. The facility will include a small animal radiation research platform (SARRP) for positioning small animals or in vitro samples, X-ray irradiation, onboard CT-imaging, and treatment planning. Additionally, there will be a microscope that enables imaging of samples during irradiation. However, since the late 1950s, the 3R Principle (Replace, Reduce, Refine animal experiments) has been established as a guideline for scientific work using animal models. This work discusses the development of a biological cancer model to be used as a preliminary stage of animal models at this pMBT facility, in order to follow this principle and reduce or replace the number of animal experiments. Compared to animal experiments, 3D models also result in easier handling, lower costs and less time expenditure.

This work shows spheroids as a 3D tumor model. The use of spheroids, is particularly beneficial in mimicking tumor behavior due to their similarity to tumors in terms of oxygen, nutrient and waste product distribution. The study of three-dimensional models comes with issues in microscopy, such as scattering and the range of the laser, which makes it hard to image the whole 3D body. These issues are mainly caused by the large size of the spheroids and the dense packing of the cells.

In this work, we discuss methods to improve the imaging of spheroids as a 3D culture model. It focuses on two basic states in which the spheroids can be studied: living and dead. Spheroid Clearing can provide better images without killing the spheroids, allowing for continued observation of the living spheroid. An alternative method would be to cut the spheroid, which would allow a complete microscopy of the spheroid, but would also definitively and irreversibly destroy it.

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