Contribution ID: **103** Type: **poster preferred**

Super resolution microscopy of ion induced repair foci in human HeLa cells

Tuesday, 16 October 2012 16:34 (1 minute)

High LET irradiation of living cells using heavy ions generates a high amount of DNA double strand breaks (DSB) in close vicinity to each other along the ion track. Various repair proteins cluster to the damage sites, e.g. γH2AX and 53BP1, and form repair foci of a gross size of about 1 µm. Due to the fact that one focus covers more than one DSB, a fine structure within the focus can be expected.
First indications for such a fine structure were found in wide field images of cells taken one hour after irradiation with 55 MeV carbon ions by a 5 × 5 µm² matrix performed at the ion microprobe SNAKE. While a typical focus with the diameter of about 1 µm can be easily resolved using a conventional fluorescence microscope, its substructures cannot be resolved due to the diffraction limit of about 250 nm in conventional fluorescence microscopy. Therefore, for analyzing foci fine structures systematically, we utilize super-resolution microscopy techniques like structured illumination microscopy (SIM), STED, or localization microscopy (SPDM) which provide a lateral resolution of about 130 nm (SIM) to 50 nm (SPDM) fwhm.
Since also with these techniques the lateral resolution is better than the z-resolution we used an irradiation configuration, where the cells are irradiated at a small-angle to the image plane. Thus, the whole ion track appears as a line within one layer of a 3D microscope image. Due to these improvements the super resolution images indicate clearly a fine structure when e.g. 53BP1 is stained with two colors. For quantification the Pearson correlation coefficient is calculated for a pixel wise shift of one color channel with respect to the other (Van Steensel approach [1]). With this, it is clear that there is a fine structure of a scale of about 200 nm. This becomes obvious by an extra correlation peak of about 200 nm FWHM. Using the same Van Steensel approach with images, where one color marks 53BP1 and the other γH2AX, we show that there is no total correlation of the fine structure between 53BP1 and γH2AX on the 200 nm scale. Using the product of the difference of the mean (PDM) 2D profiles similar 200 nm structures become visible. In addition, this approach probably show the location of multiple DSB within a single focus with high resolution when the two damage markers colocalize in small regions inside the focus but anticorrelate in larger sourrounding regions.

[1] B. van Steensel et al., Journal of Cell Science 109, 787-792 (1996)

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Session Classification: Poster Session 1

Track Classification: DNA Damage and Repair