

LONG-TERM IMAGING AND AUTOMATED IMAGE ANALYSIS APPROACH TO VISUALIZE AND QUANTITATIVELY MAP THE SPATIO-TEMPORAL DISTRIBUTION OF DNA DAMAGE FOCI WITHIN TUMOR SPHEROIDS.

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In order to maintain genomic integrity, living organisms have evolved several complex signaling pathways, collectively referred as DNA Damage Response (DDR) to protect against DNA damage events. The wealth of knowledge on mechanisms coordinating DDR and in particular DNA repair systems has been in part connected on studies that made use of live monitoring of DNA damage foci formation in 2D cell culture models. These studies have identified many actors involved in DNA repair. Currently, we lack a picture of how repair systems integrate inputs from cell's environment cues and orchestrate appropriate cellular response in 3D tissues or organs context. However, gaining insight into these processes raises important specific challenges requiring the development of approaches tailored to working with relevant biological 3D models accounting for tissue heterogeneity and environment. To reach this goal, we recently developed a methodology exploiting both live imaging of DNA damage foci formation within tumor spheroid (TS) by light sheet fluorescence microscopy (LSFM) and supervised machine learning segmentation. Extracting a quantitative 4D map (3D space plus time) of DNA damage foci formation within tumor spheroid following treatment with a genotoxic agent will require that all structures of interest be properly segmented. This is a task that can no longer be tackled manually given the sheer amount of data routinely produced by LSFM systems, and requires instead efficient computerized image processing algorithms. Consequently, we developed a computing framework based on specific object/event classifier algorithms customized for tumor spheroid images and big data sets. These tools, which utilize image processing and manual supervised machine learning (FIJI, Ilastik) enable us to detect nuclei and follow DNA damage foci highlighted by a single labeled fluorescent protein, the DNA damage sensor mDomain of 53BP1. Thanks to this powerful method, we are able to characterize and compare the distribution of DNA damage foci within the TS from both a qualitative and a quantitative point of view.

REFERENCES:

- Sommer, C. Strahle, U. Kothe, F.A. Hamprecht, Ilastik: interactive learning and segmentation toolkit, in: 2011 IEEE International Symposium on Biomedical Imaging: From Nano to Macro, 2011. pp. 230–233.
- Live cell division dynamics monitoring in 3D large spheroid tumor models using light sheet microscopy. Lorenzo, C., Frongia, C., Jorand, R., Fehrenbach, J., Weiss, P., Mandhui, A., Gay, G., Ducommun, B. and Lobjois, V. Cell Division (2011) 6 :22.