P-2.76

Unveiling Chromatin Dynamics and Dna Damage Localization Through Quantitative Analysis of Dna Counterstains

Greta Paternò [1], Elisa Longo [1], Silvia Scalisi [1], Luca Lanzanò [1]

[1] University of Catania, Catania, Italy

DNA damage is a critical factor in genomic instability, contributing to various diseases. While DNA repair mechanisms are well studied, the role of chromatin organization in the DNA damage response is less understood. To address this, we developed QUANDO (QUantitative ANalysis of DNA cOunterstains), a method using DNA counterstains to extract quantitative data on DNA damage localization and chromatin dynamics. By combining Image Cross-Correlation Spectroscopy with DNA intensity analysis, QUANDO allows assessment of DNA damage distribution in the nucleus. This technique is compatible with dual-color confocal and super-resolution microscopy. In a first application, we analyzed U937-PR9 cells, revealing that spontaneous and PML-RARα-induced DNA damage primarily localizes in euchromatin, while neocarzinostatin-induced damage is more evenly distributed across euchromatin and heterochromatin. In a second application, QUANDO was used in live-cell imaging of laser micro-irradiation-induced DNA damage in HeLa cells. Initially, damage occurred preferentially in regions of high DNA density, followed by a chromatin relaxation phase lasting few tens of seconds. We plan to investigate whether PARP1 inhibition with talazoparib impairs chromatin remodeling during repair. Finally, we aim to apply QUANDO to 3D cell spheroids to study chromatin dynamics in a more physiologically relevant model.