Automated Analysis of Colocalization in Radiation-Induced Foci

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Ionizing radiation induces a variety of DNA lesions, including DNA double-strand breaks (DSBs), which are among the most challenging to repair. The formation of these lesions initiates a cascade of DNA repair protein recruitment, leading to the formation of radiation-induced foci (RIF) such as γH2AX and 53BP1. These proteins are classical DSB markers used in fluorescent immunocytochemical staining, which reveals the number of cell nuclei containing RIF. RIF counting and colocalization analysis enable the assessment of interplay between repair proteins; specifically**,** colocalization with 53BP1 indicates the involvement of non-homologous end joining (NHEJ), one of the primary DNA repair pathways. However, quantifying colocalization is a complex task, posing challenges for both manual operator analysis and computational modeling.

To automate the detection of radiation-induced foci in fluorescent images, we utilize a two-stage deep learning pipeline: first, the pre-trained Segment Anything Model 2 (SAM2) neural network detects cell nuclei in the image; subsequently, the YOLO neural network detects RIF within each nucleus [1]. To count the number of colocalized foci (object-based colocalization), following RIF detection, local image processing is applied on each focus location. Then the contour and centroid of each focus are identified. The two foci with centroids separated by a distance less than the optical resolution of the microscope are defined as colocalized. Moreover, pixel intensity-based colocalization parameters like Pearson's correlation coefficient are evaluated for individual cell nuclei. Based on this model, we developed a web service MOSTLIT (https://mostlit.jinr.ru) for the automated detection and quantification of colocalization between two RIF populations. Validation against manual counting in normal human skin fibroblasts showed strong agreement, confirming the method's accuracy and utility.

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References

1. *Shadmehri S, et al.*. A Deep Learning Model for Automated Quantification of DNA Repair Foci in Somatic Mammalian Cells // Physics of Particles and Nuclei **Vol. 56**, No. 6, Year 2025. Pp. 1623-1627.