Tata Institute of Fundamental Research
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Webinar

Microscopy and image analysis based enhancements for investigating DNA damage responses

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In a eukaryotic cell, DNA stores genetic information, which is constantly subjected to change from various internal and external factors. Cells have evolved various mechanisms that detect and reverse these changes, maintaining information fidelity. These repair mechanisms are broadly called DNA damage responses (DDR), which are crucial for the proper functioning of the cell. Failure in DDR can lead to many cancers and neurodegenerative diseases. In this thesis, we have investigated DDR spatio-temporally in the cell with new modalities of microscopic imaging and analysis. We first developed live fluorescence anisotropy imaging combined with laser micro-irradiation to study chromatin compaction dynamics upon localised damage. Localisation and dynamics of repair factors and their correspondence to underlying chromatin structures post-damage was investigated using this method. We observed that chromatin is compacted globally upon local damage with phosphorylated forms of ATM, and PCNA forming nodes in regions of less compact chromatin, where new DNA is synthesized. Further, in these experiments we found sample size per experiment to be limiting, and I developed a software to generically improve throughput in imaging assays. With these new tools of acquisition and analysis, we vastly improve the statistics of microscopic investigations in a regular widefield setup. In terms of fixed cell immunofluorescence we can obtain data over tens of thousands of cells, and rare subpopulations can be identified in live cell dynamics. With these we observed rare cell-cycle linked post-damage events in living cells without the use of cell-cycle blockers.

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