

## **FOCUSED INDUCTION UV PHOTOPRODUCTS IN CELL NUCLEAR DNA BY THREE-PHOTON INFRA-RED RADIATION.**

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Applications based on two and three-photon processes have recently generated renewed interest because of the ability of the technique to localise the photonic interaction within a femtolitre volume. So far the two and three photon phenomena in biological studies have been applied mainly to imaging microscopy where the technique provides a unique three-dimensional detail with sub-micron resolution. The possibilities of multiphoton induced DNA damage in cells have two major implications. Firstly at certain intensities of radiation, DNA damage could occur as a collateral effect of multiphoton imaging techniques and this may invalidate experiments on live cells. Secondly highly localised induction of DNA damage in the whole viable cells is a major goal of cell biologists who wish to study DNA repair protein dynamics, individual cell signalling and cell to cell communication. We have successfully used triple infra-red (IR) photon absorption to induce cyclobutane pyrimidine dimers (CPDs) in viable cell nuclear DNA. Three-photon near-IR induction of the characteristic UV photoproducts, cyclobutane pyrimide dimers (CPDs) and 6-4 photoproducts, provides much higher spatial resolution and consequently fewer numbers of lesions. The damage which is identified by immunostaining, can be generated in a precise and distinct pattern within nanometer three-dimensional resolution ( i.e. in spot sizes of less than 300nm dimension). Induction of such very low numbers of lesions has revealed a hitherto unrecognised mobility and clustering of the non-repaired photoproducts. The movement is impaired when higher numbers of lesions are induced. The nature of this phenomenon is being explored.

The estimated intensity of the IR light which generates the 3-photon absorption in the DNA and leads to the induction of CPDs was approximately 400MW/cm<sup>2</sup>. Repair of the damage induced at this intensity was observed. We are therefore able to assess the probabilities of DNA damage induction by radiation intensities used for multiphoton imaging processes as well as induce very highly focused damage in cell nuclei.

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