

Developing a Single-Molecule Fluorescence Tool to Quantify DNA Damage

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Quantification of DNA damage is an important technique for medical physics, for example to assess damage caused by the quinolone antibiotics, or to examine the effects of novel cancer treatments such as low-temperature plasma therapy on healthy or tumorous cells. Existing damage quantification techniques such as the alkaline comet assay [1] are often subjective in their results, especially at higher damage levels. Methods using immunofluorescence [2] often lose information due to the three dimensional nature of the cell. DNA origami [3] provides a well-characterised two dimensional substrate that when used in fluorescence microscopy avoids these drawbacks.

A novel method to quantify DNA damage using single-molecule fluorescence microscopy of DNA origami has been developed. Origami tiles were dosed with low temperature plasma, then surface immobilised and labelled with the intercalating dye, YOYO-1. The total fluorescence intensity was characterised using bespoke MATLAB software [4] allowing quantification of DNA damage.

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[2]A. H. Coons and M. H. Kaplan, "Localization of Antigen in tissue cells: II. Improvements in a method for the detection of antigen by means of fluorescent antibody," *J. Exp. Med.*, vol. 91, no. 1, pp. 1-13, Dec. 1950.

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[4]H. Miller, Z. Zhou, A. J. M. Wollman, and M. C. Leake, "Superresolution imaging of single DNA molecules using stochastic photoblinking of minor groove and intercalating dyes.," *Methods*, Jan. 2015.