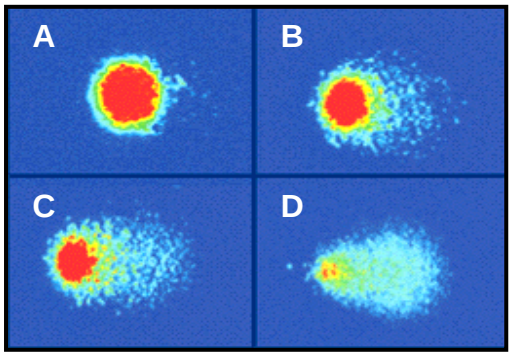
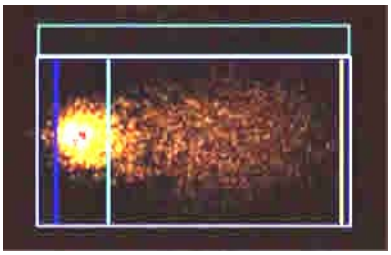
The Comet Assay

Single cell gel (SCG) electrophoresis or 'Comet assay' is a rapid and very sensitive fluorescent microscopy-based method to examine DNA damage and repair at the level of individual cells. Furthermore, the assay is cheap, has very low material requirements and can use virtually any eukaryotic cell. Since the introduction of the alkaline Comet assay in 1988, a number of advancements have greatly increased the flexibility and utility of this technique for detecting various forms of DNA damage (e.g., single- and double-strand breaks, oxidative DNA base damage, and DNA-DNA/DNA-protein/DNA-Drug crosslinking).

The assay is attractive because of its simplicity, sensitivity, versatility, speed, and economy, and the number of publications it leads to rises each year with applications in genotoxicity testing, human biomonitoring and molecular epidemiology, ecogenotoxicology, as well as fundamental research in DNA damage and repair. Comet assay not only provides an estimate of how much damage is present in cells, but what form it takes. Although it is essentially a method for measuring DNA breaks, the introduction of lesion-specific endonucleases allows detection of, for example, ultraviolet (UV)-induced pyrimidine dimers, oxidized bases, and alkylation damage.





The protocol is very straightforward. Cells embedded in agarose on a microscope slide are lysed with detergent and high salt to form nucleoids containing supercoiled loops of DNA linked to the nuclear matrix. Electrophoresis at high pH results in structures resembling comets, observed by fluorescence microscopy (see Figures A-D); the intensity of the comet tail relative to the head reflects the number of DNA breaks. The basis for this is that loops containing a break lose their supercoiling and become free to extend toward the anode. Cells containing greater levels of DNA strand break damage generate comets with more intense 'tails' (i.e. Figures C & D). Densitometric and geometric parameters of the comets are determined using image analysis software and the extent of DNA strand break damage is assessed.

As a rough guide, material costs for a single straight forward Comet Assay experiment is ~£20 per experiment and we prefer to work in collaboration.

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Some papers with Comet assay data from the Jones group:

Barber, R.C., Hickenbotham, P., Hatch, T., Kelly, D., Topchiy, N., Almeida, G.M., Jones, G.D.D., Johnson, G.E., Parry, J.M., Rothkamm, K., and Dubrova, Y.D., 2006, Radiation-induced transgenerational alterations in genome stability and DNA damage, *Oncogene*, **25,** 7336–7342.

Almeida, G.M., Duarte, T.D., Steward, W.P. and Jones, G.D.D., 2006, Detection of oxaliplatin-induced DNA crosslinks in vitro and in cancer patients using the alkaline Comet assay. *DNA Repair,* **5**, 219-225.

AL Moneef. M., Sherwood, B.T., Bowman, K.J., Kockelbergh, R.C., Symonds, R.P., Steward, W.P., Mellon, J.K. and Jones, G.D.D., 2003, Measures by the alkaline comet assay predict bladder cancer cell radiosensitivity. *British Journal of Cancer,* **89**, 2271-2276.