Localised induction of UV damage in cellular DNA for the study of DNA repair dynamics. Comparison of the methods using a focused near infra-red laser beam and a focused 248nm UV beam

W Shaikh, G J Hirst, D N Winter, S W Botchway, E Conein, R Meldrum

Central Laser Facility, CCLRC Rutherford Appleton Laboratory, Chilton, Didcot, Oxon, OX110QX, UK

Main contact email address: *r.meldrum@rl.ac.uk and r.meldrum@bham.ac.uk*

Introduction.

Localised induction of DNA damage is a major goal of cell biologists who wish to study the molecular dynamics of DNA repair [1)], intra-cellular signalling process and cell-cell communication [2)].

We have illustrated how a focused 750nm laser beam can be used to induce DNA photoproducts with nanometre three-dimensional resolution in cell nuclei in a defined geometrical pattern [2)]. Cyclobutane pyrimidine dimers (CPDs) have been induced by the focused NIR beam tuned to wavelengths 720, 730, 750, 770, 790, 810, and 830 (complete data not shown). Three photon absorption from these wavelengths would correspond to single photon absorption in the wavelength range 240nm – 280nm. The levels of CPDs induced by the different NIR wavelengths when the mean power of the laser was 10mW, is very similar and corresponds to the mode of lesion induction by 240 to 280nm single photon UV irradiation.

We have now developed methods to focus a 248nm UV laser beam onto cell nuclei to induce the DNA photoproducts in a defined pattern by single photon absorption. The different properties of the laser irradiation are likely to give rise to some different small side effects in the quality of the damage induced in the cells.

The UV irradiation is delivered in nanosecond pulses while the infra-red is delivered in femtosecond pulses. We previously observed that DNA strand breaks, under certain conditions, are induced by 248nm UV nanosecond pulses [3-6)]. Collateral one and two photon effects from the focused NIR beam are unlikely to be of significance [2)]. The intensity of the radiation used is not likely to be high enough to give rise to a significant amount of 4-photon absorption which would lead to single strand breaks.

The different distributions of damage in the cell nucleus formed by the absorption of single photon UV or 3-photon NIR are shown below (Figure 5).

It remains to be established if light scattering of UV from a single photon source is more of a problem than when 3-photon NIR is used.

Fabrication of chrome mask

The patterned mask was fabricated by removing portions of the metal layer from a commercial chrome-on-silica mask blank (see Figure 1). The 10 µm holes were made using electron beam lithography in the CCLRC Central Microstructure Facility. The broad ring was generated by excimer laser ablation using contact masking. The tube lens and microscope objective reduce the pattern size by a factor of 100 in the sample/image plane, so in the geometric optics approximation the 10µm holes are reduced to 100nm diameter.

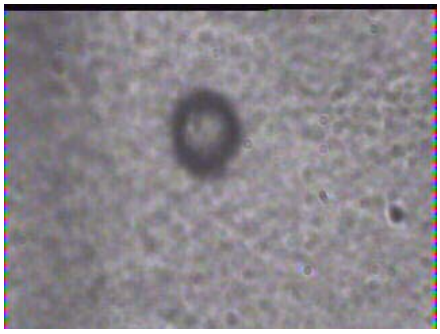

Figure 2 . Preliminary Image of UV ‘ring’, reduced by 100x, produced in homogeneous Gafchromic film – the outer ring is 10µm in diameter and 1.2µm in width.

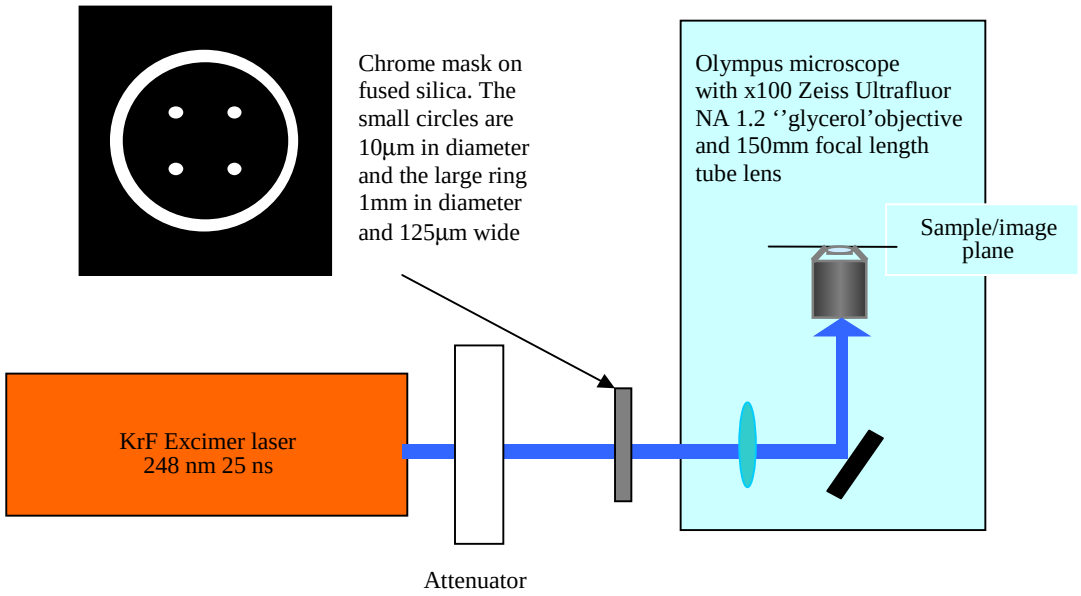

Figure 1. Schematic of UV set-up.

The active layer of this film medium is 6.5 microns – it was placed directly above a 200µm cover slip (with glycerol between the objective and cover slip) and produced using five laser shots at of 0.1mJ/cm^2.

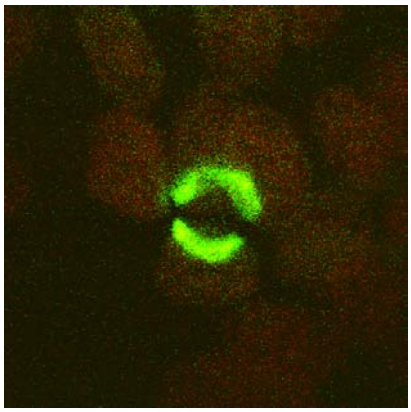

Figure 3. Pattern of DNA photoproducts induced in V79 chinese hamster ovary cells and stained with specific antibody.

Cells were grown on quartz cover-slips and irradiated as shown in the schematic diagram (Figure 1). The dose used here was a large dose (5mJ/cm^2). This overexposure has created a broadened image of the 1µm ring. The maximum width of the ring shown here is ca. 4µm. Although the cells could be viewed through the microscope eyepieces or by projection on a TV monitor, the cover-slip had to be placed manually because of a lack of a mechanical device to do this. Thus the ring covers the nuclei of two or three cells and the image is missing where there is no DNA in the path of the irradiation. Work is in progress to reduce dose levels and acquire a more faithful reproduction of the mask pattern in cellular DNA.

The important result here is that we have shown that DNA damage can be created in a defined pattern in single cell nuclei by single photon UV irradiation of cells through a microscope objective. Previously we have shown that this is possible by 3 photon absorption from a focused near infra-red laser beam tuned to a wavelength between 720 – 850nm, (Figure 4).

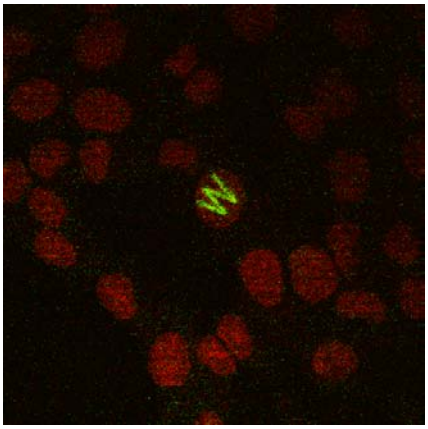

Figure 4. The W pattern outlines cyclobutane pyrimidine dimers induced by a focused 730nm NIR laser beam and stained by a specific antibody.

Distribution of DNA damage induced in the cell nucleus by single photon UV or 3-photon NIR absorption

Figure 5A shows cells which were irradiated under a mercury lamp at 254nm. The image is created by making a series of confocal scans through the depth of the cell. This is known as the z-plane image and it runs parallel to the axial direction of the incident light. The DNA damage, again detected by staining with antibody, is distributed fairly evenly through the depth of the cell with perhaps some more intense areas lying closer to the side of the nucleus on which the light first impinges.

Figure 5B illustrates how UV photoproducts which are induced in DNA by 3-photon NIR absorption are confined to the central section of the nucleus.

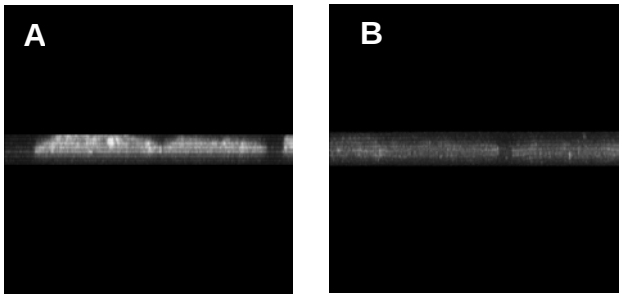

Figure 5. Z-plane images of DNA photoproducts produced in cells by single photon UV irradiation [A] and focused NIR irradiation [B].

The functions which take place in the cell nucleus, such as replication and transcription, are non-randomly distributed and their position can change throughout the cell cycle from the nuclear periphery to the centre [7]. The different distributions of damage created by the different methods used to induce the damage will have implications for the interpretation of results of biological experiments carried out using the alternative methods.

The greater challenge of increasing the resolution of the induction and imaging of the DNA damage to dimensions (ie. 200-300nm) that enable the tracking of fine movements of DNA and proteins to be studied, still remains.

Images were recorded using a Biorad Radiance 2000 laser scanning system (School of Biosciences, University of Birmingham) and a Plan Apo 60 x, 1.4 NA oil immersion lens with a working distance of 0.21mm. Z-plane scans were taken at steps of 500nm.

References

1. R.A. Meldrum, G.O. Edwards, J.K. Chipman, C.W. Wharton, S.W. Botchway, G.J. Hirst, W. Shaikh (2001) Multiphoton UV and X-ray micro-irradation to track intra- and inter-cellular dynamics. CLF Annual Report , RAL-TR-2001-030, 117 (2001)

2. R.A.Meldrum, C.W.Wharton, S.W.Botchway, G.J.Hirst, S.Topley (2002) Induction of localized UV photoproducts in cell nuclear DNA by 3-photon near infrared absorption. CLF Annual Report, RAL-TR-2002-013, 139 (2002))

3. W.Meaking, R.A.Meldrum, J.Edgerton, S.Ward & C.W.Wharton Kinetics and mechanism of DNA repair: The nature of the UV-laser damage-induced "panic' phase DNA synthesis. Ann. Rep. CLF SERC 189-191, (1993)

4. K.M.Prise, B.D.Michael, R.A.Meldrum & C.Wharton Studies of damage to plasmid DNA using 248nm laser pulses. Ann. Rep. CLF DRAL 163, (1995)

5. K.M.Prise, B.D.Michael, R.A.Meldrum and C.W.Wharton Yields of SSB and DSB in plasmid irradiated with 248nm photons: role of hydration. Ann. Rep. CLF DRAL, (1996)

6. W. Meaking (1994) PhD Thesis, Birmingham University.

7. J.B. Lawrence and G.L.Hager (2003) Nucleus and gene expression: motion meets architecture. Current Opinion in Cell Biology, 15, 255-259.