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Review

Use of near infrared femtosecond lasers as sub-micron radiation microbeam for cell DNA damage and repair studies

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ABSTRACT

Laser induced radiation microbeam technology for radiobiology research is undergoing rapid growth because of the increased availability and ease of use of femtosecond laser sources. The main processes involved are multiphoton absorption and/or plasma formation. The high peak powers these lasers generate make them ideal tools for depositing sub-micrometer size radiant energy within a region of a living cell nucleus to activate ionising and/or photochemically driven processes. The technique allows questions relating to the effects of low doses of radiation, the propagation and treatment of deoxyribonucleic acid (DNA) damage and repair in individual live cells as well as non-targeted cell to cell effects to be addressed. This mini-review focuses on the use of near infrared (NIR) *ca.* 800 nm radiation to induce damage that is radically different from the early and subsequent ultraviolet microbeam techniques. Ultrafast pulsed NIR instrumentation has many benefits including the ability to eliminate issues of unspecific UV absorption by the many materials prevalent within cells. The multiphoton interaction volume also permits energy deposition beyond the diffraction limit. Work has established that the fundamental process of the damage induced by the ultrashort laser pulses is different to those induced from continuous wave light sources. Pioneering work has demonstrated that NIR laser microbeam radiation can mimic ionising radiation via multiphoton absorption within the 3D femtolitre volume of the highly focused Gaussian beam. This light-matter interaction phenomenon provides a novel optical microbeam probe for mimicking both complex ionising and UV radiation-type cell damage including double strand breaks (DSBs) and base damage. A further advantage of the pulsed laser technique is that it provides further scope for time-resolved experiments. Recently the NIR laser microbeam technique has been used to investigate the recruitment of repair proteins to the sub-micrometre size area of damage in viable cells using both immuno-fluorescent staining of γ-H2AX (a marker for DSBs) and real-time imaging of GFP-labelled repair proteins including ATM, p53 binding protein 1 (53BP1), RAD51 and Ku 70/80 to elucidate the interaction of the two DNA DSB repair pathways, homologous recombination and the non-homologous end joining pathway.

Contents

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1. Introduction

Damage to the genomic DNA of cells may lead to severe errors in transcription and replication and if not repaired correctly may lead to mutations, genomic instability and even cell death. Earlier studies and techniques for preferential irradiation of either the cytoplasm or the nucleus [1,2] to investigate the role of DNA damage in cell inactivation showed overwhelming evidence to suggest that damage to DNA (including chromosomal damage, such as chromosome aberrations) of mammalian cells is a primary event in causing malfunction and cellular inactivation [3,4]. These experiments indicated that the nucleus is 100 times more sensitive than the cytoplasm [1] for inducing cell instability.

The development of particle radiation microbeam techniques to induce sub-cellular localised energy deposition within regions of a cell is therefore imperative to extend our understanding of cell function, in particular DNA damage and repair mechanisms [5–7]. The microbeam technique therefore allows controlling and probing more precisely nanoscale regions of damage.

Earlier methods of light induced microbeam used continuous wave or pulsed laser technology for cellular irradiations involved UV (193–355 nm) or visible (532 nm), picosecond to nanosecond sources to generate laser plasma X-rays to induce DNA damage and studies related to radiobiology [8–12]. Solution phase studies of DNA damage induction by 193 nm, 5 ns laser light results in the formation of prompt strand breaks and base modifications [13,14]. However, these methods are unsuitable for cellular studies due to the low penetration of the UV light through mammalian cells and the broad field of radiation.

There have been numerous microbeam developments worldwide based upon the construction of either low LET (linear energy transfer) ionising radiation sources (soft X-rays) with patterned grids to allow shielding of a portion of the cell nucleus to create damage and undamaged areas or high LET (alpha) sources where the transversing particle numbers are precisely controlled [15–17]. Non-ionising sources such as UV-C to UV-B (365–280 nm) within the energy range 3.94–4.43 eV have been used but these create complications for cellular studies because of competitive absorption by UV absorbing species in the electromagnetic spectrum where DNA also absorbs (Fig. 1) [17–19].

Although damage caused by low and high LET radiation include DNA strand breaks and clusters of lesions within one or two helical turns of the DNA [20], UV induces mainly base lesions, including pyrimidine dimers and 6-4 photoproducts [21]. The use of ultrafast ($<$200 fs (200 $\times$ 10^{-15} s)) lasers with less than 100 pJ of energy per pulse) have the potential to generate a plethora of DNA lesions including double-, single-strand breaks and base lesions through multiphoton absorption processes [21–25]. The mechanisms of damage involve therefore either direct ionisation or the generation of electronic excited states of the nucleic acid–base components and/or the creation of other reactive intermediates in the vicinity of DNA to induce non-direct DNA damage from water or other cellular components. A number of recent reports have reviewed generation of primarily base lesions through the multiphoton technique [25,26,27]. Due to space limitations the present review does not represent an in-depth review of the field (UV, visible, CW) and as such concentrates on the current process of DNA double strand break generation in live mammalian cells using multiphoton laser microbeam methodology, as the uses of different laser systems have previously been compared [18c,29].

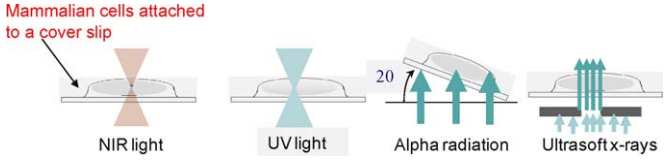

Fig. 1. Schematic diagram showing the radiation path of different sources of radiation through a mammalian cell.

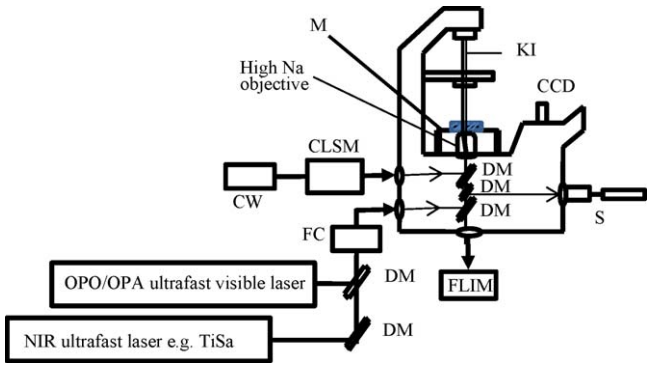

Fig. 2. Schematic of the NIR laser microbeam set up. CLSM: confocal laser scanning microscopy; Cw: continuous wave 1-P excitation lasers; DM: dichroic mirror; FLIM: advanced microscopy fluorescence life-time image; KI: Kholer illumination for white light image; M: motorised computer controlled stage; S: Spectrograph for spectroscopy.

1.1. Multiphoton laser microbeam for cellular DNA double strand break induction

Recently, we and others have developed a near infrared laser microbeam methodology coupled to confocal microscopy for irradiation of defined locations within cells together with real-time visualisation to investigate single cell DNA damage/repair dynamics [28–30] Fig. 2. In this method, the NIR irradiation induces DNA damage in individual mammalian cells through multiphoton processes that take place only within the highly focused, submicron femtolitre volume of the laser beam. The technique applies a few milliwatts (1–30 mW) of average laser power using an ultrashort (typically 50–300 fs), high repetition rate (MHz) laser light that is focused through a high numerical aperture microscope objective leading to very high peak intensities (GJ cm^{-2}) to drive the multiphoton absorption process, Fig. 3. In contrast, for continuous wave laser light sources, average laser powers of several 100 s of milliwatts are required and this may not be tolerated by the biological sample. The multiphoton absorption process was first postulated by Maria Göppert-Mayer in 1931 during her PhD thesis (Eq. (1)) [31]:

$$N_a = \frac{P_o^2 \delta}{\tau_p f_p^2} \left(\frac{A^2}{2hc\lambda} \right)^2 \quad (1)$$

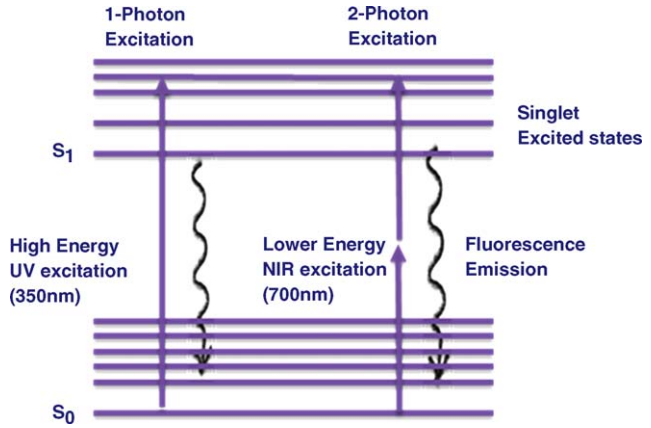

Fig. 3. One- and two-photon Jablonski energy diagram.