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Changes in Human Hair Induced by UV- and Gamma Irradiation

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Abstract

The effect of UV- and ^{137}Cs gamma radiation on the structural and chemical integrity of human hair was studied to determine the feasibility of using human hair as a non-invasive biomarker of radiation exposure to ionized gamma- and non-ionized UV-radiation. Steady state tryptophan (Trp) fluorescence and chemical analytical methods were used to evaluate the molecular integrity of Trp fluorophores and SH-groups in hair proteins and to assess the radiation induced damage quantitatively. It was found that human hair fibers were progressively damaged by exposure to both UV- and ionized gamma radiation. Damage to the hair was evidenced by a decrease in the fluorescence intensity as a result of observed depletion of the amino acid tryptophan as well as significant reduction in a number of free SH-groups in hair proteins. Hair damage was dose-dependent for exposures between 0 and 10.0 Gy and 0 - 20 J/cm^2 of UV-radiation. Additional results demonstrate that hair-fibers exposed to gamma rays, with much higher quantum energy than UV, undergo a smaller extent of changes in Trp fluorescence than when exposed to lower or equal energy of UV-irradiation. The stable Trp fluorophore appears to be extremely sensitive to UV-radiation in contrast to the ionized gamma radiation whose damage is originated from the reaction of free radicals and direct deposition of energy. We conclude that fluorescence spectroscopy represents a useful tool in the quantitative evaluation of the radiation exposure and could also be used for the rapid and non-invasive assessment of radiation dose *i.e.* biodosimeter. The approach is simple, non-invasive and appears to have considerable potential that enables quantitative evaluation of radiation dose exposure in a single hair fiber.

Keywords

Hair, Fluorescence, Radiation, Chemical Integrity

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1. Introduction

Chemical and physical properties of human hair are the subject of a remarkably wide range of scientific investigations due to their importance to the biomedical, cosmetics industry and forensic sciences. The principle protein component of hair is the cysteine rich keratin, which is composed of 18 amino acids and assembled into heavily melanized fibers that form up to 95% of hair fiber volume [1]. These protein components and structural organization of keratin contribute to most of the characteristic properties of hair.

Of the amino acids in keratin, cystine may account for as much as 24 percent. The numerous disulfide bonds formed by cystine are responsible for the great stability of keratin. On the other hand, keratin is very reactive, as cystine can easily be reduced, oxidized, and hydrolyzed [2].

Hair is very susceptible to chemical changes that occur with exposure to radiation [3]. Various abnormalities in the hair and hair follicles caused by radiation have been reported to be associated with structural re-arrangement and chemical modification in hair keratin [4]. UV-B radiation cleaves the disulfide bonds and decomposes tryptophan in hair [5]. We hypothesize that such changes might also occur upon exposure to ionizing gamma radiation and could be sensitive assays for radiation effects, and this approach could provide a basis for a non-invasive biological dosimeter [6]. While it is widely accepted that molecules of the tryptophan in the hair naturally fluoresce when illuminated, and also decompose when exposed to the ultraviolet light, their response to the ionized gamma radiation is undescribed. In contrast to skin or other cells in the body, hair fibre does not possess its own biological protective and repairing mechanisms against the impact of environmental effects. The absence of fibre regeneration makes it a potentially sensitive radiation dosimeter.

In this study, we attempt to evaluate sensitivity of Trp fluorophor to ionizing gamma radiation as well as identify key technical parameters and characteristics of fluorescent techniques that can quantify radiation-induced damage of hair. The fluorescence of this fluorophore was found to change in a predictable manner producing strong characteristic fluorescence when excited with ultraviolet light [7] [8]. This fact generates great interest for utilizing fluorescence spectroscopy to quantify damage from ionizing radiation. Such a non-invasive method that could provide a rapid and efficient assessment over time of the dose of radiation exposure would be of great value for the astronauts. Fluorescence spectroscopy is one such non-invasive technique for assessment of radiation dose utilizing hair as an accidental dosimeter following external irradiation.

2. Materials and Methods

2.1. Hair

Studies were carried out with scalp Caucasian dark brown hair collected from healthy volunteer. Single hair fiber (or bundle of two hairs) or 2 - 5 mg of hair was sufficient to make accurate quantitative analyses.

2.2. Fluorescence Spectroscopy

Fluorescence spectra in non-irradiated and in irradiated hairs were obtained with a Jobin Yvon FluoroMax-2 fluorescence spectrophotometer (Edison, NJ), equipped with a 150 W Xenon lamp, double monochromators on the excitation and emission, a photomultiplier detector (H5783P-04, Hamamatsu, Hamamatsu City, Japan). The individual hair fibers or a bundle of 2 hairs were positioned diagonally in a 1.0 × 1.0-cm polystyrene fluorometric cuvette as shown in **Figure 1** and were placed in the sample compartment of a FluoroMax-2 spectrofluorometer.

Excitation-emission spectra are constructed by measuring fluorescence emission at various excitation wavelengths, from 270 nm to 450 nm in increments of 5 nm. Emission spectra were collected starting 15 nm higher than the excitation wavelength to generate a total emission scan of 200 nm. Each fluorescence measurement consisted of a set of two serial emission spectra collected by the same positioning the hair holder. Care was taken to fix position of the hair bundle within the holder between measurements after irradiation to different dose. All fluorescence spectra were corrected for instrument response.

2.3. Gamma Irradiation

A dose response curve for low LET γ-radiation has been developed from in vitro irradiation of human hairs using ^{137}Cs Shepherd and Associates Mark I model 30 Irradiator available at NASA Ames Research Center. A

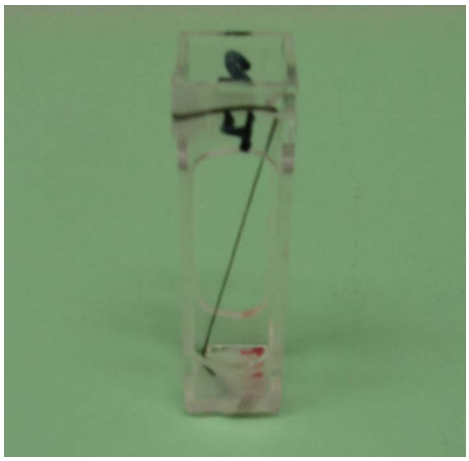


Figure 1. Positioning of hair bundle in fluorescence cuvette used in experiments. Bundle of 2 human hairs was placed in a fixed position for radiation treatment and fluorescence experiments.

dose rate was determined and exposure time was calculated from each experiment for the corresponding dose rate at one of three turntable positions with exposure rates of 0.411 Gy/min, 0.176 Gy/min, and 0.116 Gy/min respectively. The samples for the dose points were prepared in a total of 3 experiments, each with a control.

A bundle of two hairs were arranged into a disposable cuvette, which was customized for fast and reliable positioning of hair. Fluorescence characteristics of fixed hairs were first measured as non-irradiated control, and then the sample was exposed gamma radiation and changes in fluorescence spectra of irradiated sample were recorded again without changing in position of hair in cuvette. Data points for hair samples exposed to fractionated dose of radiation up to 10 Gy at 2 Gy increments were plotted to generate dose response curve.

2.4. UV-Light Irradiation

The Ultra-Violet Products Ltd., transilluminator consisting of eleven eight watt metal halide gas plasma lamps with reflecting elements has been used for UV irradiation of hair. The spectral frequencies of the light source were 279 and 295 nm. Hairs randomly positioned on the Petri dish were exposed to light from the halogen lamp at room temperature with fluencies ranging from 0 to 20 J/cm^2 at the irradiance of 7000 mkW/cm^2. The measured temperature rise in the medium was less than 3˚C during exposure to an irradiation fluency of 20 J/cm^2. UVB monitor (UVB-500C, National Biological Corp.) was used to measure the UV dose rate. A Scientech 362 power energy meter (Scientech Co., Boulder, Colorado) was utilized to measure the power of the UV-source.

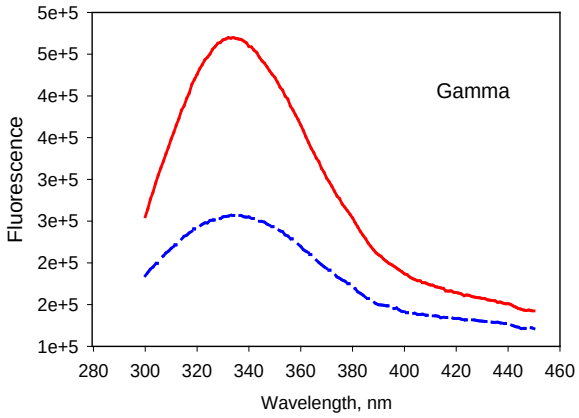
2.5. Determination of Sulfhydryl Groups

Previously described sensitive assay utilizing fluorescein mercuric acetate (FMA) for quantitative assessment of SH-groups in proteins was used to measure changes in content of sulfhydryl groups of hair keratin after exposure to UV- and gamma-radiation [9]. FMA was purified from the mono mercuric derivatives on G50 Sephadex (1.5 × 32 cm) column in solution of 10 mM [10]. Stock solution of FMA with concentration 5×10^{-4} M was stored in 10 mM NaOH at room temperature and can be used in experiments without additional purification over two weeks. Concentration of FMA was measured spectrophotometrically at 499 nm using molar extinction coefficient $E = 7.8 \times 10^{-4}$ M^{-1}·cm^{-1} [9].

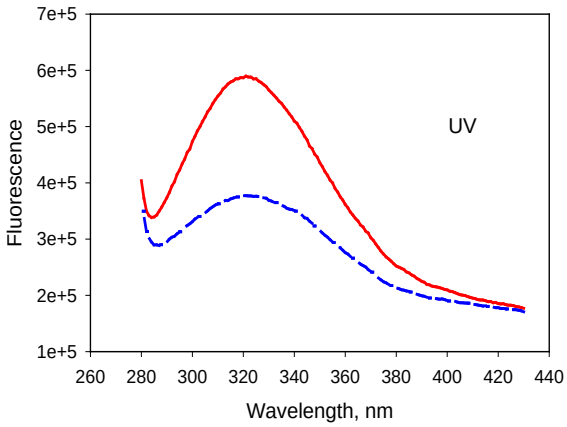
3. Results and Discussion

3.1. Effect of Gamma Radiation on Fluorescence Signal of Hair

Figure 2 shows fluorescence spectra of human hair unexposed to gamma irradiation (a) along with fluorescence



(a)



(b)

Figure 2. Changes in fluorescence spectra of hair after exposure to 10 Gy gamma and 10 J/cm^2 of UV-radiation. Solid line: non-irradiated; Dashed line: 10 J/cm^2 and 10 Gy. Resulting mean fluorescence spectra from different individuals (scalp area) before and after the irradiation.

from hair exposed to 10 Gy radiation (b). The damage to the exposed hair is demonstrated by a significant decrease in fluorescence intensity.

These changes in fluorescence are associated with tryptophan that absorbs UV light at 290 nm and emits fluorescence at 340 nm, indicating a degradation of this amino acid. For both irradiated and non-irradiated hairs, the signal changed during spectra recording was noticed and the exposure of the samples to light was therefore minimized.

These results demonstrate that hair may serve as a robust indicator of radiation exposure and impact of the radiation on hair can be quantified with fluorescence spectroscopy. A calibration curve can be established to determine the degree of damage from various dose of radiation.

Figure 3 shows data points for hair samples exposed to fractionated dose of radiation up to 10 Gy at 2 Gy increments plotted to generate dose response curve.

The average changes in hairs fluorescence induced by gamma irradiation, obtained by pooling the fluorescence data of 3 hair samples, are presented as a function of radiation dose and the error bars represent standard deviations within the studied population.

To evaluate the equation of dose-response curves, the number of hair samples was examined at different doses, and dose-response curve of the induced MN was obtained by fitting the linear-quadratic model $y = a + bD + cD^2$, where y is the changes in fluorescence of hair, a is the spontaneous yield, b is the coefficient of the one-track component, c is the coefficient of the two-track component, and D is the dose in Gy. When plotting on a linear

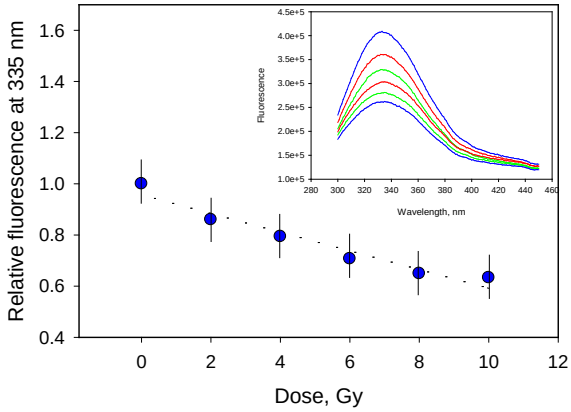


Figure 3. Dose dependence of hair fluorescence in response to gamma irradiation as measured at 335 nm. Insert show changes in fluorescence spectra of human hair exposed to different doses of gamma irradiation (0; 2; 4; 6; 8; 10 Gy).

scale against radiation dose, the line of the best fit was $Y = 0.004 + (1.882 \times 10^{-2} \pm 9.701 \times 10^{-5}) D + (1.43 \times 10^{-3} \pm 1.571 \times 10^{5}) D^2$, ($R^2 = 0.9996$) after the gamma exposure. There was a significant relationship between the change in tryptophan fluorescence of hair and dose. The dose-response curves were linear-quadratic. These data show trends towards decreasing of hair's fluorescence with increasing dose.

3.2. Effect of UV-Radiation on Fluorescence Signal of Hair

Figure 4 shows changes in the fluorescence intensity of human hair after exposure to different doses of UV-radiation. Damage to hair fibers from various exposure time of UV radiation presented as dose response curve is indicated by decrease in fluorescence intensity with increasing radiation dose. After a non-linear, preliminary stage, hair shows a linear dose-response curve to UV-radiation in a range from about 10 J/cm^2 to 25 J/cm^2, although the signal is already detectable below 1 J/cm^2. The best fit of the entire dose response curve shows that it exhibits linear-quadratic behavior.

Repetitive recording of the fluorescence spectra of the hair progressively resulted in decrease spectra intensity providing evidence on accumulative degradation of Trp after each spectra recording.

In order to confirm differences between impact of UV- and gamma irradiation on structural and chemical properties of hair as obtained from fluorescence measurements, quantitative analysis of keratin's SH-groups was performed. **Figure 5** shows changes in the content of sulfohydryl group in keratin exposed to different dose of UV- and gamma radiation.

Exposure to radiation resulted in a progressive increase in the number of free SH-groups on UV irradiated hair in amount proportional to the decrease in fluorescence intensity. In contrast to UV-irradiation, the ionized radiation has little effect on the number of free SH-groups in hair. This is in agreement with the tryptophan fluorescence measurements, which reveals that hair responds differently to UV- and gamma radiation in terms of chemical modification of tryptophan. The UV-sensitivity of sulfur groups and tryptophan of hair keratin is higher than that for the gamma radiated hairs. Although our results are consistent with the general understanding of the effect of the gamma ray and UV-light exposure on hair, we find that damaging impact of radiation do not correlated with considering amount of absorbed radiation energy. A gamma ray emitted by an atom of Cesium 137 has an energy of 0.662 million electron-volts or 10.592×10^{-14} J. (1 MeV is 1×10^6 eV and 1 eV = 1.6022×10^{-19} J). The energy of light with a wavelength of 280 nm is 7.099×10^{-19} J as calculated using Equation $E_\lambda = hc$. This is almost 5 orders of magnitude less than energy of gamma radiation. In the case of UV radiation of hair we have shown that changes in tryptophan fluorescence and number of SH-group are disproportionally larger in a case of UV radiation than an equal energy of gamma radiation. It might be caused by the presence of the sensitive targeting hot spots, which are less sensitive to the energy of radiation but more sensitive to the density of the radiation energy as it might be in the case of UV- and gamma-radiation. The effectiveness of the produced changes depends upon the structure of the polymer and the experimental conditions of irradiation such as energy and fluence.

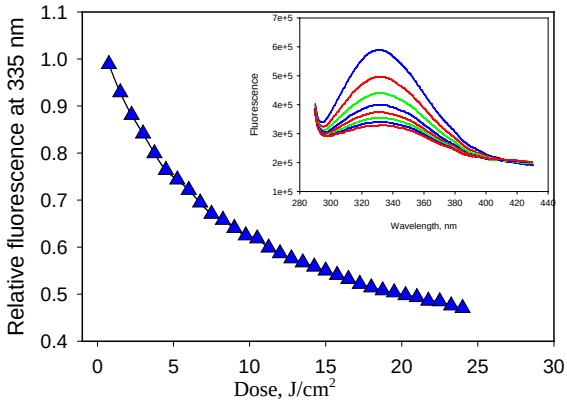


Figure 4. Dose dependence of hair fluorescence in response to UV-irradiation as measured at 335 nm. Insert show changes in fluorescence spectra of human hair exposed to different doses of UV- irradiation (0.225; 4.5; 6.75; 9.0; 11.28; 13.5 J/cm^2).

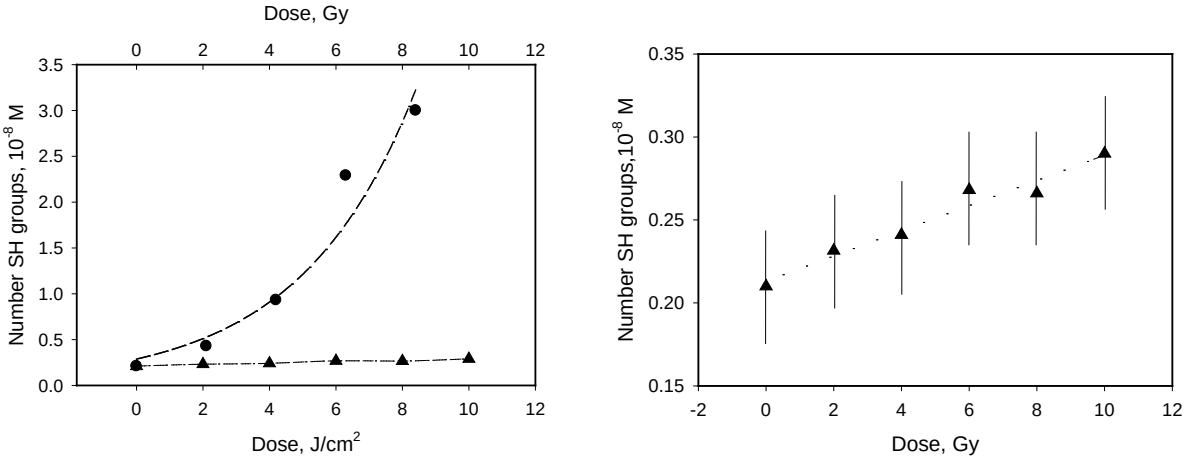


Figure 5. Changes in content of SH- groups of human hair exposed to different doses of gamma-irradiation (a). Comparison of changes in content of SH- groups induced by UV- and gamma irradiation (b).

Another difference between gamma radiation and UV is that UV radiation has predominant damaging effect on the wavelength at which material best absorbs while gamma radiation is non-specific. In the case of UV, the damage is done by photons absorbed directly by tryptophan. Photons absorbed by other components of the target do not contribute to the critical damage. In contrast, the damage by gamma for such specific target as tryptophan is less effective per amount of energy deposited but it can affect other targets in hair's keratin. In addition, up to 70% of damaging effect of ionizing radiation arrives from the secondary effect of the radiation, therefore, damage to hair's tryptophan is not mostly due to direct deposition of gamma energy, but to free radicals generated through ionization of water or other components of the target. Therefore the damaging effect of ionizing radiation is significantly reduced in dehydrated biological materials such as hair that has only between 10% - 30% of water compared to 70% of water in biological tissues [11].

The difference in character of the dose response curves for UV- and gamma radiation can be explained by not only the specific energy absorption by tryptophan but also by limited number of available targets for non-penetrated UV radiation (tryptophan) in hair keratin. Previous studies of the fluorescence characteristics of the hair suggested that the fluorescence from the tryptophane contributed significantly to the 340 nm band as a correlation was observed between the fluorescence intensity of that band and the number of tryptophanes in UV-damaged hair [12]. What makes the dose response curve steeper at the low UV-radiation doses, is the sufficient number of intact tryptophans per the targeting surface of hair. This number is progressively decreases with time

(or dose) of radiation exposure and the dose response curve becomes less steeper at the high UV doses when no more intact tryptophans are available. In contrast to non-penetrated UV-radiation, penetrated gamma radiation targets not only tryptophans located on the surface of hair but also the tryptophans hidden inside, so "absorption per unit mass" is appropriate. When significantly higher number of tryptophans is damaged, this leads to depletion of the tryptophane fluorescence signal at higher doses of gamma radiation exposure. It can be predicted that at the sufficiently high doses of UV- and gamma radiation when all tryptophans will be damaged, dose response will become flat, and not responsive to detect further radiation-induced damage.

While the depletion of tryptophane fluorescence in response to ionized radiation closely resembles that of the UV irradiated samples, UV- and gamma radiation may damage hair through different mechanisms. It is reasonable to expect that changes in tryptophan fluorescence associated with UV radiation is due to its specific absorption in the UV light, while the radiation induced damage to the tryptophane residues in human hair due to the gamma irradiation is less specific.

In addition to the depletion of the amino acid tryptophan, we have consistently detected a progressive increase in the number of free SH-groups on UV irradiated hair in amounts proportional to the decrease in fluorescence intensity. The increasing amount of free sulfhydryl groups (-SH-) in keratin can originate from disruption of the disulfide cysteine bonds (-SS-) induced by radiation. The breaking of all of these disulfide bonds in cysteine, may results in the amount of cysteine equals 720 μmol/g of feathers [13]. Data presented in **Figure 5** show that after exposure to 10Gy gamma radiation a sulfohydril group content in hair keratin change insignificantly from 0.21 μmol/g in non-radiated hair to 0.29 μmol/g in irradiated hair, which indicates that only less than 10% of the disulfide bonds were broken. Therefore, according to these criteria the native keratin was not significantly modified. Exposure to the UV-radiation change sulfhydryl group content from 0.21 μmol/g in non-radiated hair to 3.0 μmol/g in hair exposed to 10 J/cm^2, which is substantially higher than in a case of gamma irradiation. Therefore, in contrast to UV irradiation, the ionized radiation has little or no effect on the number of free SH groups in hair. The disruption of the disulfide bridge nearby the tryptophan residue by UV light excitation of this aromatic residue has been reported in previous studies [14]. Tryptophan excitation energy disrupts a neighboring disulfide bridge, which in turn leads to altered structural integrity and stability. While the Trp residues are certain "hot spot" for the UV radiation, facilitating disruption of S-S bridges, this might be not the case for the ionized gamma radiation whose damage originates from the reaction of free radicals and direct deposition of energy. In fact, it has been demonstrated that disruption of S-S crosslinks in hair induced by radiation may occur not with SH groups as an end product, but rather through the oxidation leading to the formation of cysteic acid, $CySO_3H$. This may explain the lack of reduced SH-groups in the gamma-irradiated hair in contrast to the UV-irradiation.

The discrepancy between delivered dose and damaging effects of ionizing and non-ionizing radiations on hair may also be derived from the difficulties involved in relating UV doses to gamma ray. At the same time, there are several publications demonstrating that biological effect of UV-radiation in dose range 10 - 20 J/m^2 can be significantly more damaging than of 7 or 10 Gy gamma irradiation [15] [16].

3.3. Potential Application

Since we have observed a direct correlation between the total delivered dose of ionized radiation and specific features of the fluorescence spectra it would be desirable to identify features leading to application of this property for the potential design of a sensitive biological dosimeter. The ability of a human hair to predict radiation dose throughout a window of time represents an important advance in the development of non-invasive dosimetry. In a large-scale radiologic emergency, estimates of exposure doses and radiation injury would be required for individuals without physical dosimeters. Current methods are inadequate for the task, so we are developing simple fluorescence-based approach for radiation dosimetry. This approach could provide both an estimate of physical radiation dose and an indication of the extent of individual injury or future risk.

We demonstrated that a portable fluorometer based on Ocean Optics Inc. software and fiberoptics is capable of quantitatively evaluating the dose of radiation exposure in one hair fiber, and could be used for the rapid and non-invasive assessment of radiation dose, *i.e*. bio-dosimeter. We anticipate doing further investigation to determine if tryptophan can also serve as a sensitive marker for the ionizing radiation to measure the total dose of radiation exposure using fluorescence spectroscopy.

We have repeated some of the florescence measurements on human hair utilizing both bench-top FluoroMax-2 fluorescence spectrophotometer and miniaturized optical unit produced by Ocean Optics (**Figure 6**).

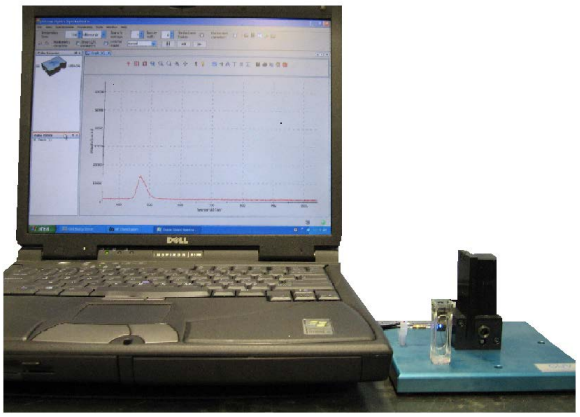


Figure 6. Ocean Optics Inc. Fiberoptics Prototype for portable device estimating dose exposure of hair using fluorescence marker. The picture represents an actual miniaturized fluorometer attached to a Dell laptop. In order to take a radiation exposure dose reading, a human hair could be fixed in to a cuvette after which the cuvette would be inserted in to the fluorometer where it would be read in live time.

Results on estimation of absorbed dose were consistent with those obtained using bench-top fluorometer demonstrating that portable miniaturized version of fluorometer can be used for assessment of radiation dose received by astronauts during space mission without using a sophisticated radiation dosimeter.

Our use of human hair for evaluating the biological effects of radiation could be helpful in predicting the extent of DNA damages in different scenarios, namely after an occupational, environmental, accidental or medical exposure. However, it may depend on gender, age and life style. Our particular interest is the follow-up study of astronauts during long-term spaceflight mission on International Space Station (ISS). With this approach it would be possible to easily detect biological effects of doses in case of unexpected solar and galactic radiation events. Such capability could be crucial in making life saving medical decisions for exposed astronauts and for determining other health consequences.

Future efforts in the area of applied hair-based bio-dosimetry will be on developing customization to market available miniaturized fluorometers and selecting the proper instrument for hair measurement such as solid state fluorescence that may fill in the gaps of the current assay for spaceflight applications. Two particular parameters are important in selecting the proper instrument for this hair evaluation: a) data should be collected quickly, to be able to process a large number of samples; b) Instrument should be compact, fully automated, and simple for users to operate. It would be advantageous if the instrument can be equipped with a fiber-optic accessory, so human hair can be positioned and fixed for examination.

Apart from steady state fluorescence spectrometry used in this study other hair analyzing fluorescence techniques such as solid-state fluorescence should be evaluated and compared for consistency of signal in hair analyses.

Further studies are needed to better control the uncertainties involved. The fluorescence signal dependence with hair qualities such as color, thickness, growth rate and treatments should, for example, be addressed. Variation based on preparation of the hair prior to analysis, *i.e*. the positioning procedure, also needs to be investigated further. To our knowledge, there have been no previous studies on the fluorescence measurements of hair following gamma radiation exposure. Hence, the dynamics behind dose of exposure and subsequent fluorescence signal are, to a large extent, unknown.

Acknowledgements

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