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Safety Analysis: Relative Risks of Ultraviolet Exposure from Fluorescence Spectroscopy and Colposcopy Are Comparable*

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ABSTRACT

Fluorescence spectroscopy is a promising tool for use in the diagnosis of disease in human tissue. However, few published reports have evaluated the safety of this technique, despite the fact that many spectroscopic systems use UV illumination. This study determined the relative risk associated with light exposure from spectroscopic systems compared with the traditional light sources that are used to illuminate tissue and direct biopsies. We compared spectroscopic detection systems for the cervix to the colposcope, a low-power microscope routinely used to illuminate the cervix, which does not cause any known photochemical damage. We measured the average spectral irradiance (W/[cm²nm]) and the average tissue exposure time during a diagnostic colposcopy examination. To quantify the relative risks, we multiplied illumination spectra by several action spectra from the literature and compared the areas under the curves corresponding to each procedure. The risk associated with the average power colposcope served as our basis for comparison. We conclude that the risks of illumination using spectroscopic systems are lower than or comparable to those already encountered in routine diagnostic procedures such as colposcopy with an average power colposcope. Spectroscopic examination can be associated with a somewhat higher risk than a colposcopy with the lowest power colposcope or a shorter than average colposcopy. The analysis presented can be repeated to estimate the magnitude of risks associated with other spectroscopic diagnostic devices.

INTRODUCTION

The use of optical spectroscopy for diagnosis of disease in human tissue appears promising. Several studies have successfully demonstrated the use of elastic scattering (1), fluorescence (2–6), infrared absorption (7) and Raman spectroscopy (2,8,9) for disease diagnosis in various organ systems. Auto- and dye-induced fluorescence have shown promise in the detection of atherosclerosis and various types of cancers in the breast (2), colon (4,10), bladder (5), lung (6) and cervix (11–13). These spectroscopic systems may offer some important advantages compared with present diagnostic practices. Currently, diagnosis is not immediate; a definitive precancer or cancer diagnosis requires analysis of a tissue biopsy. In several organs, the physician must use a specially designed white light source to visualize the tissue. The physician looks for areas suggestive of cancer and performs biopsies where appropriate. Because this examination is subjective, extensive training is generally required. New spectroscopic systems have been designed that could reduce the need for clinical expertise and could yield diagnostic information in near real time, which would help in targeting areas for biopsy. Once algorithms are extensively verified, some lesions could be followed without biopsy.

While preliminary clinical results obtained with fluorescence spectroscopy are encouraging, the presence and degree of any risk to the patient as a result of illumination with quasimonochromatic light at various wavelengths have not been rigorously examined. Damage to tissues due to this illumination could occur *via* two mechanisms: photochemical and photothermal. In this paper, we examine the possible photochemical risks associated with a spectroscopic detection system. Many spectroscopic diagnostic devices based on fluorescence and elastic scattering use UV radiation, which is known to damage DNA, primarily through induction of thymine dimers (14). Exposure to solar UV radiation is also a risk factor for cataract formation (15) and malignant melanoma (16). Although ANSI standards are available to calculate skin and eye limits to laser radiation (17), it is unclear whether mucosal epithelial tissues possess the same mechanisms for repairing UV-induced damage as does the skin.

A plot of the efficacy of damage production as a function of wavelength is called an action spectrum. The action spectrum is dependent on the particular measure of damage studied; results have been reported for dimer production in DNA in solution (18) and in cultured cells (14), cell cytotoxicity in cultures (15), tumor development in animal models (19) and induction of DNA–protein crosslinks in human cells (20). The UVB region of the spectrum, from 290 to 315 nm,

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is more efficient at dimer production than is the UVA region of the spectrum, from 315 to 400 nm (14), and thus an analysis of the relative risk of a spectroscopic system must take into account the illumination energy as a function of wavelength.

In this paper we present a method for comparing the relative risk of spectroscopic systems to those of standard methods of diagnosis that also use light. We illustrate this method for several spectroscopic systems we have developed for use in the detection of precancerous cervical lesions. This method of comparison can be repeated by investigators to estimate the exposure levels, risks and safety limits of spectroscopic diagnostic instruments being developed for other organ sites.

MATERIALS AND METHODS

Summary. To compare the relative risk of light exposure from a traditional white light source and the spectroscopic imaging systems, we first measured and compared the spectral irradiance (W/[cm^2 nm]) from each. The exposure times used with each system were used to calculate the spectral radiant exposure (J/[cm^2 nm]). Finally, the spectral radiant exposure of each was multiplied by several action spectra at each wavelength, and the areas under these curves were compared to assess the relative risks of the techniques. Three spectroscopic systems were evaluated: (1) a system that illuminated a single pixel of tissue with a pulsed laser, (2) a system that illuminated multiple pixels of tissue with a pulsed laser, and (3) a system that illuminated multiple pixels of tissue with a continuous wave (CW) xenon lamp.

Spectroscopy systems. As described in detail previously (21), the single-pixel pulsed-laser excitation system incorporates a nitrogen-pumped dye laser, an optical fiber probe and an optical multichannel analyzer. It was used to record fluorescence spectra of the cervix *in vivo*. The laser was fired with a 30-Hz repetition rate and the pulse duration was 5 ns. We assumed a laser bandwidth of 0.5 nm. A 0.785 mm^2 area of cervical tissue was excited at 337, 380 and 460 nm with the following average transmitted pulse energies and number of pulses: 8.71, 7.60 and 13.95 μJ/pulse, and 10, 50 and 50 pulses, respectively. The transmitted spectral radiant exposures were 2.2 E-2, 9.7 E-2 and 1.8 E-1 J/(cm^2 nm), respectively.

The multipixel pulsed-laser excitation system, described in detail elsewhere (22), is similar to the single-pixel system above, but it illuminates a larger total tissue area, 0.785 cm^2, at only one wavelength. At 337 nm, the laser pulse energy was 385.6 μJ/pulse, and the laser was operated at 10 Hz with an exposure time of 6 s. We again assumed a laser bandwidth of 0.5 nm. The resulting spectral radiant exposure incident on the tissue was 5.91 E-2 J/(cm^2 nm).

The third spectroscopy system, a multipixel CW xenon lamp excitation system currently in development, is similar to the second, with the source replaced with a filtered xenon lamp. A 20 nm-bandwidth filter centered at 337 nm was first used, followed by 10 nm-bandwidth filters centered at 380 nm and 460 nm. The spectral irradiance was 1.28 E-4, 4.9 E-4 and 8.5 E-4 W/(cm^2 nm), at the center wavelengths 337, 380 and 460 nm, respectively. Exposure times of 3 s for each excitation wavelength resulted in spectral radiant exposures of 3.85 E-4, 1.47 E-3 and 2.55 E-3 J/(cm^2 nm), respectively, for the same center wavelengths.

Measurements of colposcope output. During colposcopy, the cervix is illuminated with white light for an extended period of time. We measured the spectral irradiance incident on the cervix from eight colposcopes (Leisegang Medical Inc., models 1B3 and 1H3) in the M. D. Anderson Cancer Center Colposcopy Clinic. The radiant power at 460 nm was measured from an additional five colposcopes. For each colposcope, the measurements were made with both its current light bulb and with a new light bulb, to account for variability in the light bulbs as well as in the colposcopes. We also measured the average time the cervix was illuminated during the colposcopy procedure in a series of 20 colposcopy patients in the Colposcopy Clinic. The distance between the colposcope and the tissue is always adjusted to bring the tissue into clear focus. At this focal distance, the spot size of the colposcope is approximately 5.7 cm^2, which is large enough to illuminate the entire cervix. Therefore, the average time of the colposcopy procedure is also the average time of cervical exposure. The colposcope radiant powers were measured at their focal planes with a detector with a smaller area (1.27 cm^2) than the colposcope spot size. Therefore, irradiances were later computed by dividing the measured colposcope powers by the area of the detector head.

The spectral irradiance (W/[cm^2 nm]) was determined in a two-step process. The first step involved measuring the shape of the spectral output. The colposcope light was coupled *via* a fiber-optic probe into a spectrograph (Chromex 250 IS), which then dispersed the light onto a cooled charge-coupled device (CCD) camera (Princeton Instruments TE/CCD-576 EW) under computer control. The colposcope spectrum was found to be much higher in the visible region of the spectrum than in the UV region. Because the UV portion of the spectrum contributes most significantly to the relative risks, an experiment was performed to determine the lowest wavelengths at which UV radiation could reliably be measured from the colposcope. The concern was that, due to limited out-of-band rejection of the spectrograph, stray light from the visible region of the spectrum may contribute to the measured values in the UV and result in an overestimation of the true level of UV radiation. To determine the amount of UV radiation being emitted from the colposcope, the spectrum was measured and then remeasured using a series of filters. Filters were selected that transmit light in the UV region of the spectrum but block light from the visible wavelengths (UG5, UG11, Schott). The UG5 filter only and then both the UG5 and UG11 filters were inserted into the light path, at which time the intensity values (corrected for the filter transmission characteristics) obtained in the UV region became constant. The minimum peak-to-peak signal-to-noise ratio at 320 nm exceeded 25:1, but the signal-to-noise ratio at 310 nm was only 10:1. The colposcope spectrum was then represented as a composite of the spectra measured with combinations of the filters (320–450 nm) and that measured without any filters (above 450 nm). The spectra were corrected for the transmission characteristics of the UG filters.

To correct for the nonuniform spectral response of the measurement system, correction factors were calculated from the measured spectra of two different spectral irradiance standards. The correction factors obtained with a deuterium arc lamp (UV standard for spectral irradiance, Optronics OL UV-40) were used to correct the colposcope spectrum from 300 to 400 nm. An NIST traceable calibration tungsten ribbon filament lamp (Optronics 550C) was used to calculate correction factors for the colposcope spectrum above 400 nm (23). The standard lamps and the colposcopes were measured using the same instrumentation. Two standard lamps were used because the tungsten lamp output in the UV spectral range is very low compared to that in the visible spectrum and stray light is again a concern. In this way, the true shape of the colposcope spectral output was determined.

We measured the absolute power from one colposcope at nine different wavelengths (310, 337, 380, 420, 440, 460, 490, 500 and 520 nm) using a calibrated power meter (Newport 1815) with a UV-sensitive calibrated photodiode (Newport 818-UV). Each of the filters had a 10 nm bandwidth except for the 337 filter that had a 20 nm bandwidth. Each wavelength was selected by inserting a bandpass filter into the light path, and power values at each wavelength were corrected for the bandwidth and transmission of the corresponding filter by dividing by the area under the appropriate filter transmission spectrum. When measuring the power at UV wavelengths (310 and 337 nm), additional filters (UG5, UG11) were used to block stray light as discussed above. The repeatability of individual power measurements was tested and found to be within 10%.

We compared the shape of the colposcope spectrum obtained with these power measurements to that obtained with the spectrograph by calculating the ratio of the absolute power in calibrated units to the corresponding intensity measured earlier in arbitrary units. There was about 10% random variation in this ratio for the eight wavelengths ranging from 337 to 520 nm, and we attribute this variation to power measurement repeatability. However, the ratios calculated using the 310 nm filter were consistently lower than the others by 30–40%, indicating that, below 315 nm, lack of out-of-band rejec-

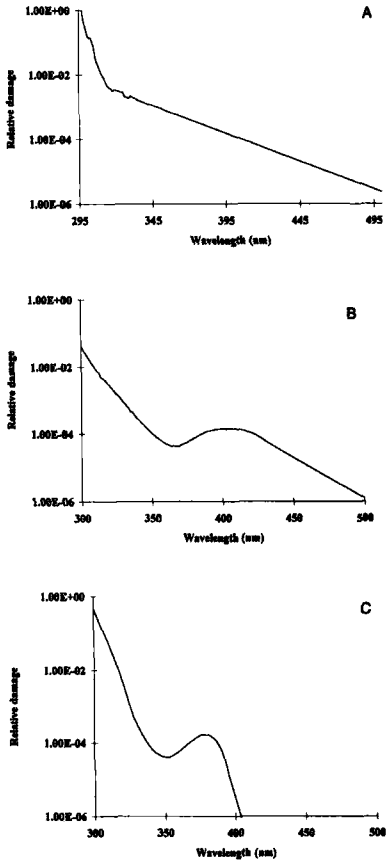

Figure 1. Comparison of the action spectra for (a) cytotoxicity in cultured lens epithelial cells (15), (b) protein–DNA crosslinks in human cells (20) and (c) induction of skin carcinogenesis in mice (19). The action spectrum in a has been extrapolated from 365 to 495 nm and the action spectrum in c has been extrapolated from 395 to 500 nm.

tion is significant in the measurement of the colposcope spectrum. Because this variation at wavelengths other than 310 nm is so small, power values measured only at 460 nm were subsequently used to convert the magnitude of the colposcope spectrum from units of detector counts to spectral radiant power (W/nm). Because the ratio calculated at 310 nm was not in good agreement with those calculated at the other wavelengths, and due to problems with out-of-band rejection at wavelengths below 320 nm, we concluded that only the UV radiation measured at wavelengths above 320 nm should be included in our risk analysis.

An average colposcope spectrum was calculated to be used in the risk analysis. The spectra measured from eight different colposcopes were normalized and then averaged to yield a spectrum with a representative shape. The power measurements of 13 colposcopes, with old and new bulbs, taken at 460 nm were averaged and used to scale this average spectrum to units of W/nm. We thus determined the average colposcope output in calibrated power units over the entire wavelength range of interest. To convert this colposcope spectral radiant power (W/nm) to spectral irradiance (W/[cm^2 nm]), each colposcope spectral radiant power was divided by the area of the UV detector head, 1.27 cm^2. The minimum and maximum colposcope spectra were similarly calculated by scaling the normalized, average spectrum with the minimum and maximum powers measured at 460 nm.

Estimation of measurement uncertainties. Given our method of measurement, there are two types of errors possible: errors in the lineshape of the colposcope spectrum and errors in the overall magnitude of the colposcope output. Possible sources of error in the lineshape include lack of out-of-band rejection, nonlinearity of response of the multichannel detector, errors in the calibration of the spectral standard lamps used and noise associated with the multi-

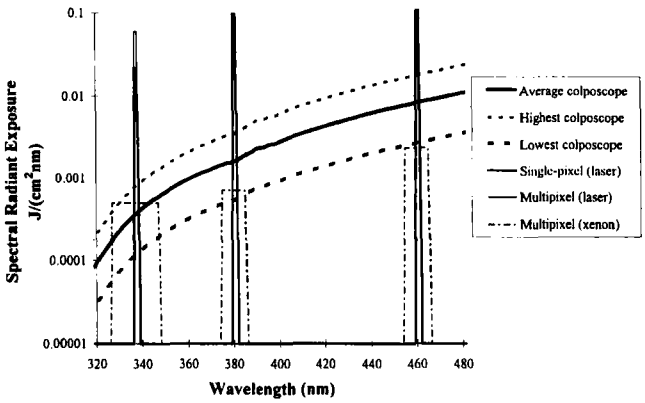

Figure 2. Spectral radiant exposure for each spectroscopic system tested and for the lowest, average and highest power colposcopes. Note that the y-axis is a log scale.

channel measurements. Possible sources of error in the magnitude of the colposcope output include calibration uncertainties in the power meter, alignment of the power meter with respect to the colposcopes and noise associated with the power meter measurements. We have quantified the magnitude of each source of error, either by measurement or from instrument specifications. The dominant source of error in the measurement of the magnitude of the colposcope output is the 10% error associated with the power meter noise and alignment. The dominant source of error in the lineshape measurements is lack of out-of-band rejection, which, above 320 nm, could not be measured above the error associated with the power meter measurements. Assuming all errors were independent and could be added in quadrature, the total uncertainty in our measurements is approximately 11%.

Computation of relative risk. We multiplied each of the illumination spectra by action spectra found in the literature and compared the areas under these curves to quantify the relative risk associated with each procedure. For all three action spectra, the integration limits were 320–500 nm. The action spectra for cell cytotoxicity and mouse skin carcinogenesis were extrapolated from 365 nm and 395 nm, respectively. However, whether these curves are extrapolated or cut off sharply has little effect on the resulting risk values and does not affect our conclusions. The risk associated with the average-power colposcope was used as our basis for comparison and was arbitrarily assigned a value of 1. The action spectra of Fig. 1, for cell cytotoxicity of cultured lens epithelial cells, DNA–protein crosslinks in human cells and animal skin carcinogenesis, were chosen after completing a literature search using the key words action spectrum, UV radiation and DNA damage. Over the past 30 years, we found only about 20 papers that were relevant to our current study. Several research groups authored many of the papers, and most focused on UV-induced damage to the eye, skin or DNA. We selected the three action spectra in Fig. 1 because they covered the wavelength range of interest and were representative of the type of action spectra available.

RESULTS

Colposcopes

We measured the spectral output of eight different colposcopes and the power output at 460 nm from a total of 13 colposcopes, with both new and old light bulbs. The spectral shapes of the colposcopes varied only slightly, but the most remarkable difference between colposcopes and light bulbs was the intensity. Figures 2 and 3 include the minimum, average and maximum power colposcopes to illustrate the range of results. The average time of illumination during a colposcopic examination, t, was 7.1 min with a standard deviation of 5.4 min. The product of spectral irradiance

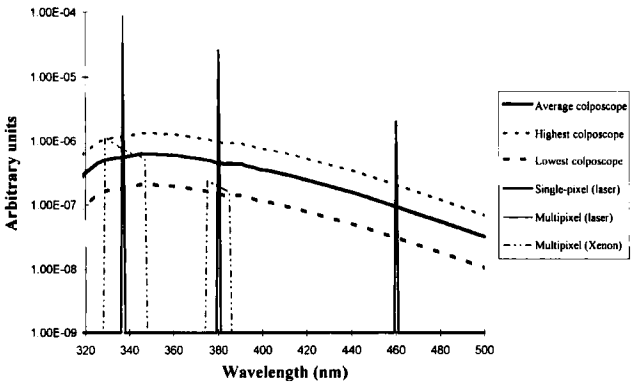

Figure 3. The product of the action spectrum (15) and the spectral radiant exposure for each spectroscopic system and for the lowest, average and highest power colposcopes. Note that the y-axis is a log scale.

(W/[cm^2 nm]) and t yields the spectral radiant exposure of the cervix during the average colposcopy procedure. Figure 2 shows the spectral radiant exposure as a function of wavelength for each spectroscopic system and the lowest, average and highest power colposcopes.

Spectroscopy systems

For the single-pixel spectroscopic system, the spectral radiant exposure delivered to the cervix was at least an order of magnitude greater than that from the average-power colposcope for the wavelength bands centered at 337, 380 and 460 nm. The second spectroscopic system, a multipixel laser system, only illuminates the tissue at 337 nm, and the spectral radiant exposure delivered at this wavelength was two orders of magnitude higher than that of the average-power colposcope at 337 nm. The spectral radiant exposure of the multipixel xenon lamp spectroscopic system was approximately the same as that of the average-power colposcope in the illumination band centered at 337 nm. However, the spectral radiant exposure delivered by this spectroscopic system was approximately half and one-fourth of those of the average-power colposcope in the bands centered at 380 nm and 460 nm, respectively. This comparison indicates that, in the wavelength bands to be illuminated with the spectroscopic systems, the spectral radiant exposure delivered to the cervix varies from being much lower to much higher than that received during typical colposcopy.

Assessment of risk

Figure 3 shows a representative result of the multiplication of the illumination and action spectra for a single action spectrum, that for cell cytotoxicity (15). Table 1 compares the risk for an average-length colposcopy (7.1 min) with the lowest, highest and average-power colposcopes to those of the spectroscopic systems based on the three action spectra of Fig. 1.

The relative risk of the low-power colposcope was three times lower than that of the average-power colposcope, while the maximum-power colposcope was associated with a risk of approximately two times that of the average colposcope.

The relative risk of spectroscopic examination with the single-pixel system was lower than or comparable to that of a colposcopy with the average-power colposcope. When the DNA–protein crosslink action spectra were used, the relative risk was also comparable to that of a colposcopy with the lowest power colposcope. However, when the other two action spectra were used, the risk associated with this system was greater than that of a colposcopy with the lowest power colposcope. The risk associated with the laser multipixel system was also lower than or comparable to that of a colposcopy with the average-power colposcope and was comparable to that of a colposcopy with the lowest power colposcope when the DNA–protein crosslink and mouse skin carcinogenesis action spectra were used. When the cytotoxicity action spectrum was used, the risk associated with this system was higher than that of a colposcopy with the lowest power colposcope. The relative risk of spectroscopic examination with the xenon multipixel system was lower than that of a colposcopy with the average-power colposcope and comparable to that of a colposcopy with the lowest power colposcope for all three action spectra considered. Based on this comparison, we conclude that the risks of illumination using these spectroscopic systems are lower than or comparable to those already encountered in an average colposcopic examination.

Table 1. Comparison of the relative risks of the different systems based on the integral of the product of each energy curve and action spectrum*

System	Cytotoxicity action spectrum, Fig. 1a	DNA–protein crosslink action spectrum, Fig. 1b	Mice skin carcinogenesis action spectrum, Fig. 1c
Lowest power colposcope	0.33	0.33	0.33
Average-power colposcope	1.00	1.00	1.00
Highest power colposcope	2.16	2.16	2.16
Single-pixel (laser)	0.56	0.28	0.95
Multipixel (laser)	0.82	0.36	0.33
Multipixel (xenon)	0.32	0.17	0.42

*The results for the average-power colposcope have been normalized to 1 for comparison purposes.

DISCUSSION

Colposcopy does expose cervical tissue to UV radiation, and we have not analyzed the absolute risk associated with this procedure. However, colposcopy is being performed as a routine diagnostic tool, and we are not aware of any reports of photochemical damage due to its illumination, despite widespread and long-standing use of this clinical technique. We have shown that, based on the DNA–protein crosslink formation, cell cytotoxicity and mouse skin carcinogenesis action spectra, the spectroscopic systems we tested are safer than or comparable in risk to a typical colposcopy examination.

As shown in Table 1, while the risk associated with the spectroscopy systems is always comparable to or lower than a colposcopy with the average-power colposcope, there are some instances where the spectroscopy systems are associated with a higher risk than a colposcopy with the lowest power colposcope. In addition, if a colposcopy examination was shorter than average, our analysis would show that a spectroscopic examination can be associated with a somewhat higher risk than colposcopy. However, it is important to remember that colposcopy is a routine diagnostic procedure and that no deleterious effects have been associated with it in over 40 years of routine clinical use. While patient comfort is always a concern, the length of a colposcopy is determined by the expertise of the colposcopist, the complexity of the case and whether it occurs in a teaching setting involving many physicians.

Other potential biological effects of UV radiation, important both in spectroscopy and colposcopy, are not considered here. These include activation of the herpes simplex virus (HSV), human immunodeficiency virus (HIV) and human papillomavirus (HPV). Exposure to UV radiation is a known risk factor for induction of recurrent HSV disease in animals and humans, but the mechanism involved is poorly understood (24,25). The overall effects of UV radiation exposure on HIV infection in human beings are also unknown, and the literature offers no clear recommendations to HIV-infected patients regarding this issue (25–28). The effects of UV radiation on HPV transcription are unknown, and this is an important area for future research (29,30). Despite these potential areas of concern, colposcopy is routinely done on women who are HPV positive, HPV and HIV positive and HSV positive with no adverse effects noted by clinicians.

This study addresses two important issues. Fluorescence spectroscopy is emerging as a diagnostic tool for several organ sites, and investigators must utilize the current knowledge of UV-induced tissue damage to minimize the risks to their patients. In addition, UV radiation has been shown to activate the viruses discussed above, but appropriate quantitative relationships have not yet been reported for such effects. Therefore, further studies of these effects are warranted so that action spectra covering the wavelength range of interest can be calculated and this information can be used more effectively when estimating safety limits of UV radiation exposure. Based on current knowledge alone, we should design instruments that expose tissue to the minimum required amount of UV radiation, while remaining aware of the ongoing research being done in this important area.

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