Human Skin Auto-fluorescence Decay as a Function of Irradiance and Skin Type

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ABSTRACT

The aim of this work was to establish measurement conditions under which endogenous skin fluorescence ("auto-fluorescence") is relatively invariant, so that changes in exogenous agents can be accurately determined. Fluorescence emission was measured on the volar forearm of 36 subjects, chosen to be equally representative of all 6 Fitzpatrick skin types. All subjects were exposed to approximately 40 minutes of optical excitation at 450 and 500 nm with 4 irradiances between 0.3 and 9 mW/cm^2. Both non-optically-induced (e.g. tissue settling and fluctuation) and optically-induced variations were observed in the measured fluorescence and mechanisms explaining these effects are proposed. The optically-induced auto-fluorescence decay was independent of skin type when excited at 450 nm, but significantly dependent on skin type when excited at 500 nm. Further, the extent of decay over time was linearly related to irradiance at 500 nm, but at 450 nm was non-linear, with the extent of decay rolling off between 2 and 9 mW/cm^2. In order to maintain the auto-fluorescence signal within 95% of its original value over a 30 minute period, the excitation at 450 nm would need to be limited to 1.5 mW/cm^2, while excitation at 500 nm should be limited to 5 mW/cm^2.

Keywords: Auto-fluorescence, endogenous fluorescence, photo-bleaching, fluorescence decay, human skin, Fitzpatrick skin type, irradiance dependence, power dependence

1. INTRODUCTION

Diagnostic optical measurements made through human skin often rely on the assumption that the skin is relatively un-perturbed by the optical interaction. For example, in order to accurately measure the fluorescence of an exogenous agent in the skin, the endogenous skin fluorescence needs to be stable and well-characterized. Prior studies have demonstrated skin auto-fluorescence is susceptible to photo-bleaching when exposed to sufficiently high irradiances at visible excitation wavelengths.[1-3] Other studies have demonstrated that visible light can induce skin darkening on both short and long time scales.[4] However, prior studies have not systematically addressed the effect of skin type along with variation in irradiance and excitation wavelength.

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2. METHODS

2.1 Instrumentation

Two fluorescence instruments were constructed for this study, one supplying excitation at 450 nm, and a second at 500 nm. The 450 nm instrument employed a temperature-stabilized diode laser (40 mW, Power Technology Inc. model number LDCU8/7577), that was optically chopped at 1.6 kHz (Stanford Research Systems, model number SR540), and split 4 ways before being coupling into four separate bifurcated fiber optic probes (Newport, model number 77565) to provide skin surface irradiances of 0.3, 0.8, 2.5, and 8.7 mW/cm^2. The fiber optic bundle consisted of a randomly-mixed close-packed array of the source and detection fibers (individual fiber diameter: 100 μm, 0.22 NA, common bundle diameter: 3.2 mm). The four bundles were linearly arranged with a center-to-center spacing of 3.2 cm, so that illumination from all 4 bundles could be simultaneously applied to the forearm of each subject. Each bundle was placed directly behind a 2 mm thick, 7.5 mm glass (BK7) window that was embedded flush within a black plastic (ABS) block, used to support the subject's arm from below. The light diffusely reflected within the skin and re-collected by the fiber optic probe was collimated (Newport part number 77644) and filtered (488 nm long-pass, Semrock LP02-488RS-25; 560 nm center wavelength with 25 nm bandwidth, Semrock FF01-560/25-25), and detected using a photomultiplier (Hamamatsu model number H7827-001). The detected signal was digitized and amplified using a lock-in amplifier (Stanford Research Srystems, model number SR830) referenced to the chopper frequency.

The 500 nm instrument employed two LED sources, one providing the highest irradiance (Lightspeed model number HPLS-36AD3500) and a second source (Thorlabs model number LEDC9) that was split 3 ways, to provide the 3 lower irradiances. The LED sources were electronically amplitude-modulated at 1.6 kHz and band-pass filtered with 500 nm center wavelength and 24 nm band-pass (Semrock part number 500/24) in order to limit overlap with the fluorescence emission wavelengths. Fluorescence emission was long-pass filtered with a 532 nm cutoff (Semrock part number LP03-532RS-25), and band-pass filtered with 593 nm center wavelength, and 40 nm band-pass (Semrock part number FF01-593/40-25). The detectors used for the 500 nm instrument (Hamamatsu part no. H7827-011) had enhanced red-sensitivity compared to those used in the 450 nm instrument. The 500 nm system used the same type of fiber optic bundles and the same amplification and digitization scheme as the 450 nm system.

2.2 Study Protocol

Light exposure from the four fiber optic bundles at 450 nm was simultaneously applied to right arm of each subject; while the left arm was used for 500 nm exposure. The exposure of the 2 arms was performed sequentially. The position of the 4 fiber bundles on each arm was randomized on a subject-by-subject basis in order to prevent correlation of irradiance variation with arm position. Subjects were seated next to each test station with their forearm resting on top of the optical sampling block throughout each ~40 minute measurement period. The measurement protocol consisted of 50 seconds of baseline collection (no illumination), followed by 300 seconds of light exposure, followed by 200 seconds of dark collection (no illumination), followed by 1800 seconds of light exposure. Fluorescence emission measurements were collected continuously at 1 Hz frequency throughout the protocol.

Subjects ranged in age between 18 and 57, and were equally divided according to gender and according to 6 skin type categories. Skin type was characterized according to the Fitzpatrick scale,[5] with 1 corresponding to the lightest colored skin, and 6 the darkest. The fluorescence decay curves of several subjects showed large spurious deviations that appeared to correlate with subject movement (gesturing, sneezing, etc.). In these cases, the entire decay curve was excluded from the analysis. The study was performed in accordance with an Institutional Review Board (IRB) approved protocol.

3. RESULTS

Figures 1a-d show fluorescence emission traces excited at 450 (Figures 1a-b) and 500 nm (Figures 1c-d), averaged across the subjects with the lightest (Figures 1a and 1c) and darkest (Figures 1b and 1d) skin color. The four traces within each figure correspond to different optical irradiances (0.3-9 mW/cm^2). Significant fluorescence decay was observed in virtually all subjects and at all irradiance levels. Not all of the decay is optically induced, as is apparent by comparing the fluorescence intensity before and after the period in which the light source was blocked ("dark period", between 350 and 550 seconds). This was particularly apparent in subjects with lighter colored skin. For example, looking at the top trace (lowest irradiance) in Figure 1a (excitation at 450 nm, skin type 1), the fluorescence decay is well-approximated by a single exponential function, with the dark period included in the fit. Similarly, with excitation at 500 nm (Figure 1c), the fluorescence decay clearly continues even when the light source is blocked. In contrast, in subjects with the darkest skin color, the fluorescence decay does not consistently continue during the dark period, and in some cases even recovers to some extent during the dark period. For example, see the lowest trace (highest irradiance level) in Figure 1b.

In addition to the overall decay of the fluorescence traces, both periodic and non-periodic structure was observed within the individual fluorescence traces. This structure had no significant contribution from instrumentation noise, as was verified by studies using the same apparatus to measure the fluorescence in an invariant sample (a stable dye-filled cuvette) over the same time period. Instead, the structure is associated with properties of the tissue being probed (and will be hereafter referred to as "tissue noise"). The amplitude of this tissue noise was generally observed to be larger when excited at 450 than 500 nm (for example, compare Figures 1a-b with Figures 1c-d). The amplitude of the tissue noise was estimated by fitting the overall decay of the lowest irradiance (0.3 mW/cm^2) traces to a single exponential, and subtracting the resultant fit from the data. Averaged over all subjects, the standard deviation of residual traces was two times greater for 450 nm excitation (2.2%), compared to 500 nm excitation (1.1%). A periodic component to the structure was clearly observed in approximately one third of the subjects. Fourier transform power spectral analysis revealed the peak frequency to be between about 50 and 100 mHz (3-6 beats per minute). The peak frequency varied by subject, but within a given subject was generally consistent across excitation wavelength and irradiance level. The phase of the periodic variations was observed to be dependent on the subject's arm position.

The percent change of the fluorescent intensity after 40 minutes at the lowest irradiance was averaged across each skin type, and is summarized in Figures 2a-b. The error bars represent the standard deviations across the subjects within each skin type. Assuming that optically-induced fluorescence decay is minimal at this irradiance level (justified below), the effect is apparently caused by the mechanical interaction between the forearm and the optical probe, and will hereafter be referred to as a "settling" effect. The settling effect decreases with increasing skin type at both excitation wavelengths, with the overall extent of settling at 500 nm being approximately 25% higher than at 450 nm excitation.

In order to separate the settling effect from optically-induced decay (hereafter referred to as "photo-decay"), for each subject and excitation wavelength, the decay curve collected at the lowest irradiance level was used to normalize the three decay curves at higher irradiance levels. The results, averaged across all subjects, are summarized in Figures 3a (450 nm excitation) and 3b (500 nm excitation). Only results subsequent to the dark period were included in this analysis. With 450 nm excitation, photo-decay is evident even at the 0.8 mW/cm^2 irradiance level, and is well-characterized by a single exponential model having a decay constant of approximately 550 seconds. Photo-decay induced at the 2 highest 450 nm irradiance levels (2.5 and 8.7 mW/cm^2) required a 2 exponential model, with one decay constant shorter than 100 seconds, and a second decay constant longer than 500 seconds (see Figure 3a for actual fitting parameters). With 500 nm excitation, no photo-decay was evident at the 0.9 mW/cm^2 irradiance level. At the two highest 500 nm irradiances, a two exponential model was again required to fully capture the shape of the decay curve.

The irradiance dependence of the photo-decay at 450 nm excitation is clearly non-linear, as illustrated in Figure 4a. The percent photo-decay rolls off at irradiance levels above about 2.5 mW/cm^2. This is the case whether the photo-decay is measured at early (200 seconds) or late (2000 seconds) in the subject exposure. The percent photo-decay observed when exciting at 500 nm is linear over the same range of irradiances. In order to maintain the auto-fluorescent signal within 95% of its original value over a 30 minute exposure time, Figures 4a and 4b show that the irradiance should be kept at or below about 1.5 and 5 mW/cm^2, for excitation at 450 and 500 nm, respectively.

The photo-decay effects were also averaged according to skin type, and the percent of photo-decay after 2000 seconds is summarized in Figures 5a-b. No significant dependence on skin type was observed for the 450 nm excitation wavelength (Figure 5a). However, for 500 nm excitation at the highest irradiance level, the percent of photo-decay clearly increased with increasing skin type (Figure 5b).

4. DISCUSSION

In addition to the expected photo-induced decay of the skin auto-fluorescence, significant decay of the fluorescence was observed even during dark periods. The clear dependence of this effect on skin color suggests that it originates at depths greater than the melanosomes (located within the epidermis). We hypothesize that this settling effect is due to pooling of blood within the dermal layer, as the subject's arm rests on the optical sampling platform. In darker-skinned subjects, the melansome layer strongly absorbs the excitation light, largely confining the optically sampled area to shallow depths containing little pooled blood. The observed 25% greater settling effect at 500 nm excitation, compared to 450 nm, is consistent with this hypothesis, since light at 500 nm will have a greater penetration depth into the skin, and thus greater potential for interaction with the blood-containing (dermal) layers.

Following this same hypothesis, the tissue noise observed within the fluorescence decays could be explained as local oscillations in blood content caused by periodic opening and closing of surface blood vessels. The greater (~2x) magnitude of the tissue noise observed at 450 nm excitation, compared to 500 nm, at first glance appears to be in contradiction with the settling results. In order to explain the observations, the hypothesis needs to be elaborated, with a blood-pooling layer residing deeper in the skin (deep in the dermis), compared to a more shallow-lying layer of oscillatory blood vessels (closer to the dermal-epidermal border). The greater tissue noise amplitude observed in dark-skinned compared to light-skinned subjects is also consistent with the effect originating at shallow skin depths, since the melanosome layer will confine photons to more shallow depths in these subjects. The agreement in frequency but difference in phase between different skin locations measured simultaneously on the same subject, suggests a model in which shallow blood vessels open and close as a wave moving across the skin with a frequency in the range of 50 to 100 mHz.

After taking steps to remove the settling effect, it can be seen that the remaining optically-induced fluorescence decay observed with 450 nm excitation is independent of skin type (Figure 5a), whereas the decay induced by 500 nm excitation clearly increases with skin type (Figure 5b). The lack of dependence on skin color at 450 nm suggests that the photo-decay occurs at depths more shallow than the melanosomes (within the epidermis). The fact that significant photo-decay is observed even at low (e.g. 0.8 mW/cm^2) surface irradiance, is also consistent with the target chromophore lying at a shallow depth, where the excitation light has had little chance to diffuse. Prior work has also suggested the presence of an easily photo-bleached chromophore in the top layer (stratum corneum) of the epidermis.[2] This component was also previously suggested to be associated with the shorter time constant term in 2-exponential fits to decay data.[2] However, the present results do not agree with this prior assignment, since the same non-linear power dependence is observed on both short (200 second) and long (2000 second) time scales.

The significant skin-type dependence of the photo-decay induced by 500 nm excitation suggests that the target chromophore lies within or deeper than the melanosomal layer. The weaker dependence on irradiance at 500 nm, compared to 450 nm, is also consistent with the target chromophores being located in a deeper layer of the skin. The observation that the photo-decay increases with skin type is puzzling if attributed to photo-bleaching of a chromophore within the dermis, such as Collagen. Instead, the observed dependence on skin type suggests that the melanosomes themselves are participating in the photo-decay, either by photo-bleaching, or by increasing their absorbance in response to light at 500 nm. This type of rapid visible-light-induced skin darkening has been previously documented.[4] In the darkest-skinned subjects, the effect also appears to be rapidly (within minutes) reversible (see, for example, the lowest trace in Figure 1b).

5. CONCLUSIONS

In order to accurately measure the fluorescence of exogenous agents in the skin, the fluorescence due to endogenous agents needs to be stable and well-characterized. At least 3 sources of auto-fluorescence variation were observed in this study: (1) A settling effect, that continued even during dark periods, and is hypothesized to be due to mechanically-induced pooling of blood deep within the dermis, (2) Tissue noise, that is highly variable from subject-to-subject, with a periodic component at 50-100 mHz in about one third of the subjects, that is hypothesized to be due to opening and closing of small blood vessel near the dermal/epidermal interface, and (3) Photo-decay that has a non-linear dependence on 450 nm excitation in the irradiance range of 0.3 to 9 mW/cm^2, but linear dependence for 500 nm excitation, and is independent of skin type for 450 nm excitation, but positively correlated with skin type when excited at 500 nm. Evidence suggests that the primary target chromophore for the 450 nm photo-induced effect lies at shallow depths within the epidermis. The primary target chromophore for the 500 nm photo-induced effect is hypothesized to be melanin, with the "photo-decay" actually due to a photo-induced absorption increase by the melanosomes.

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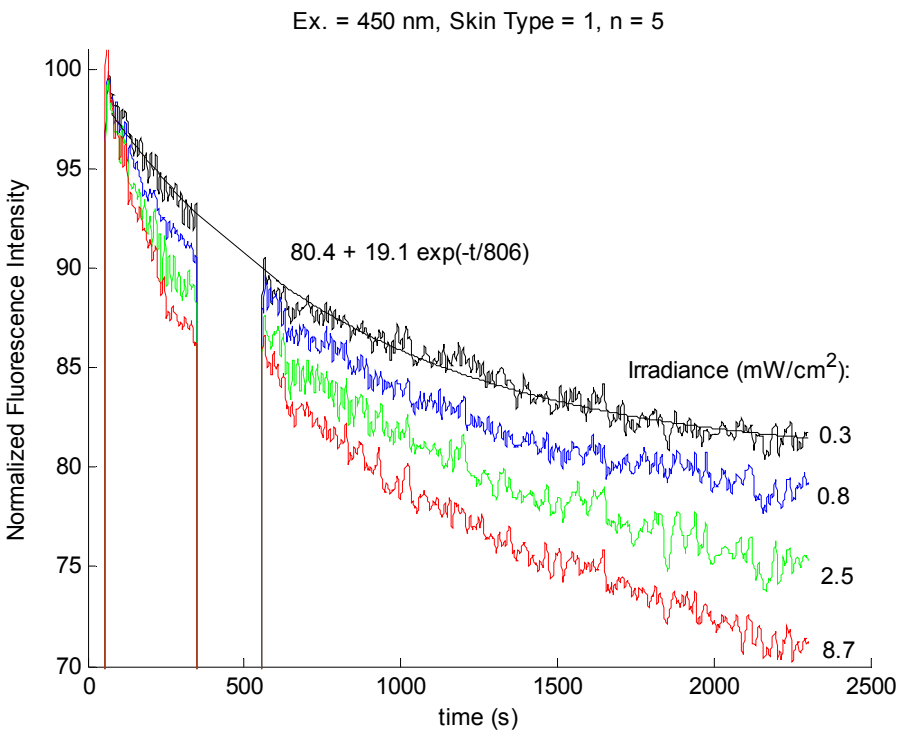

Figure 1a. Normalized fluorescence decay as function of irradiance, excitation: 450 nm, Skin Type: 1.

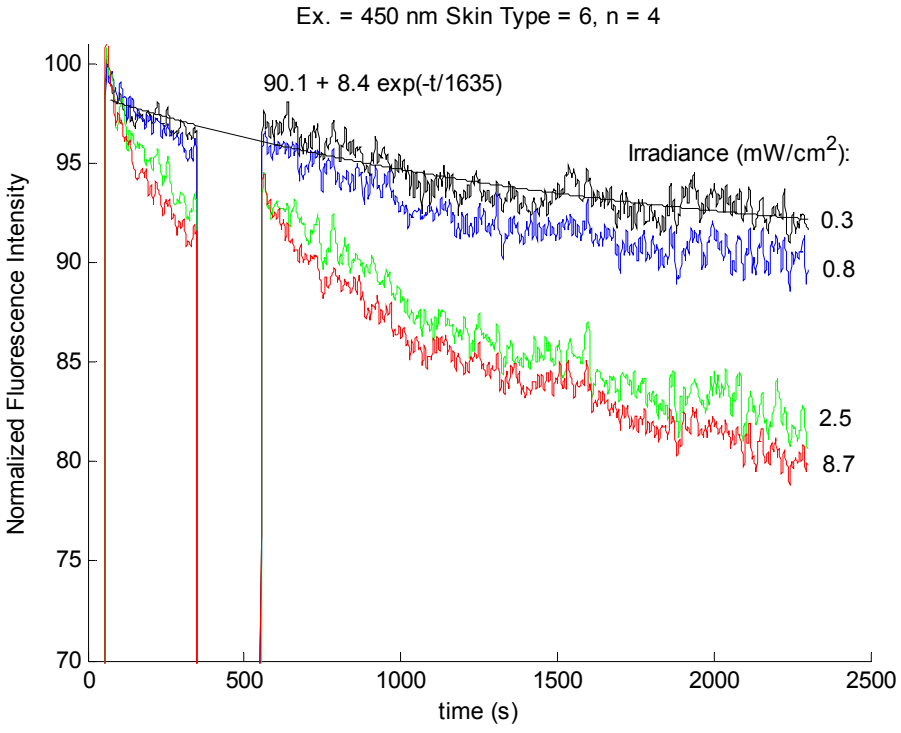

Figure 1b. Normalized fluorescence decay as function of irradiance, excitation: 450 nm, Skin Type: 6.

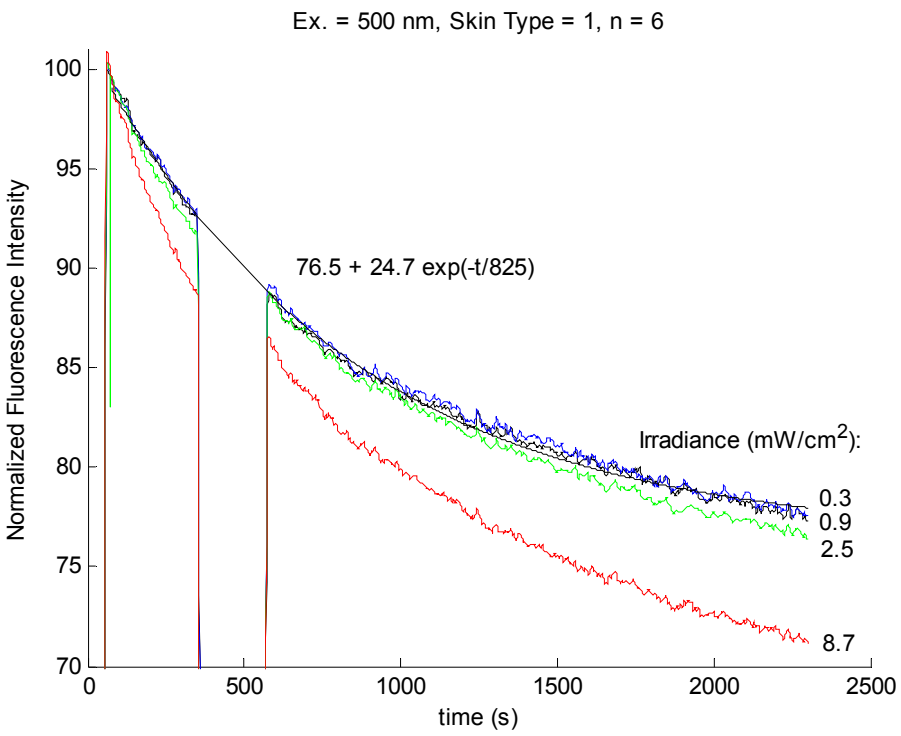

Figure 1c. Normalized fluorescence decay as function of irradiance, excitation: 500 nm, Skin Type: 1.

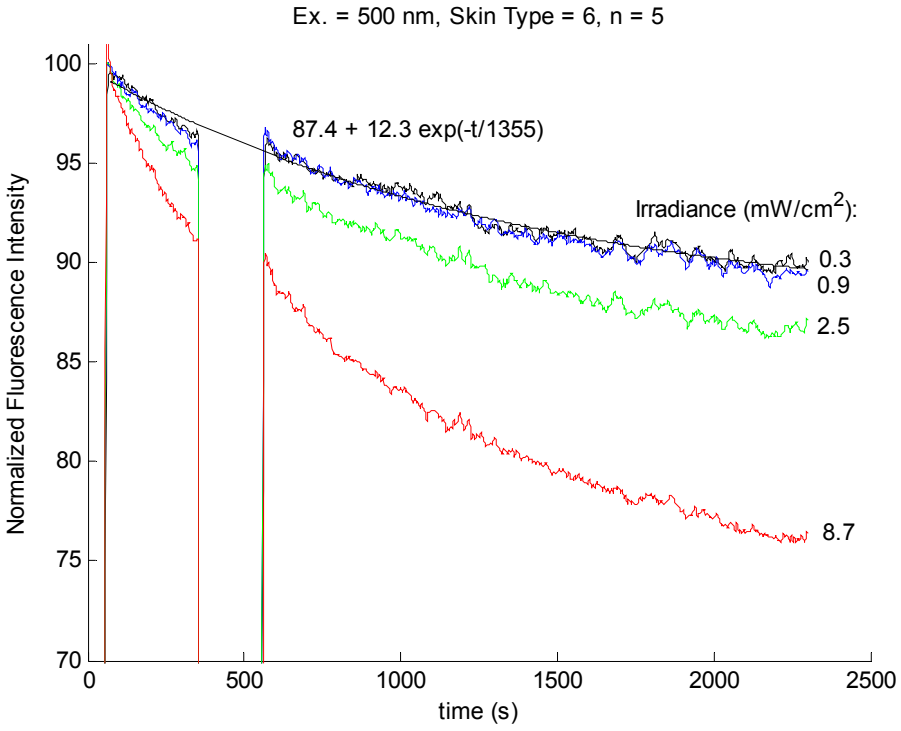

Figure 1d. Normalized fluorescence decay as function of irradiance, excitation: 500 nm, Skin Type: 6.

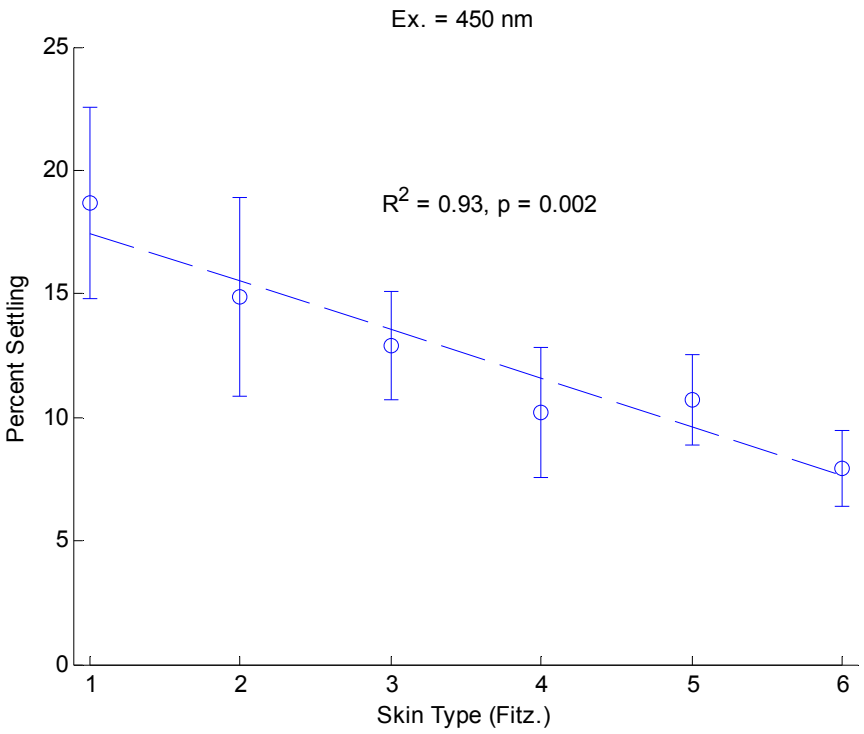

Figure 2a. Percent settling as a function of skin type. Excitation: 450 nm.

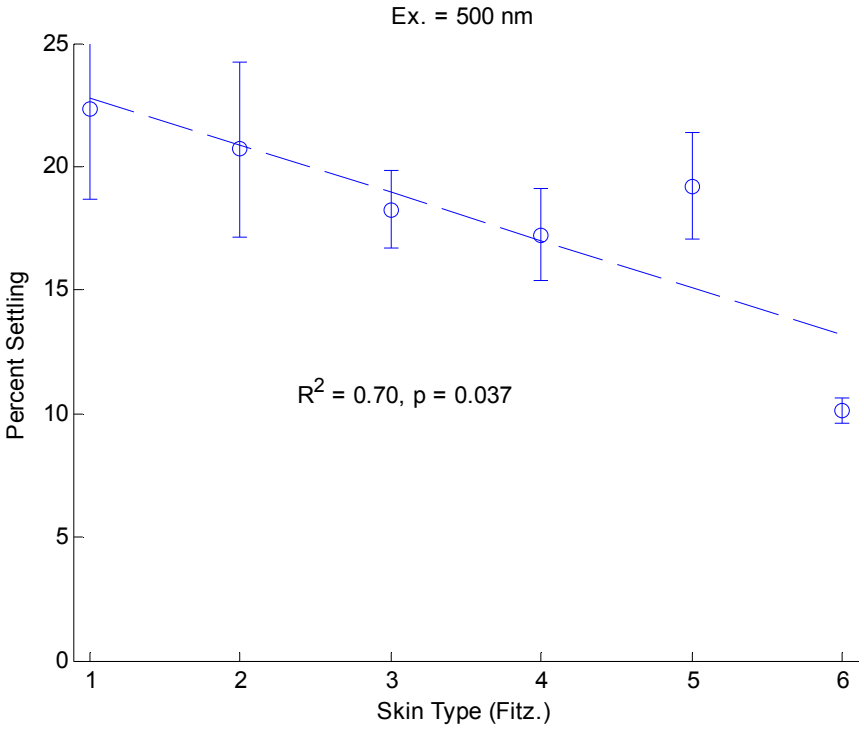

Figure 2b. Percent settling as a function of skin type. Excitation: 500 nm.

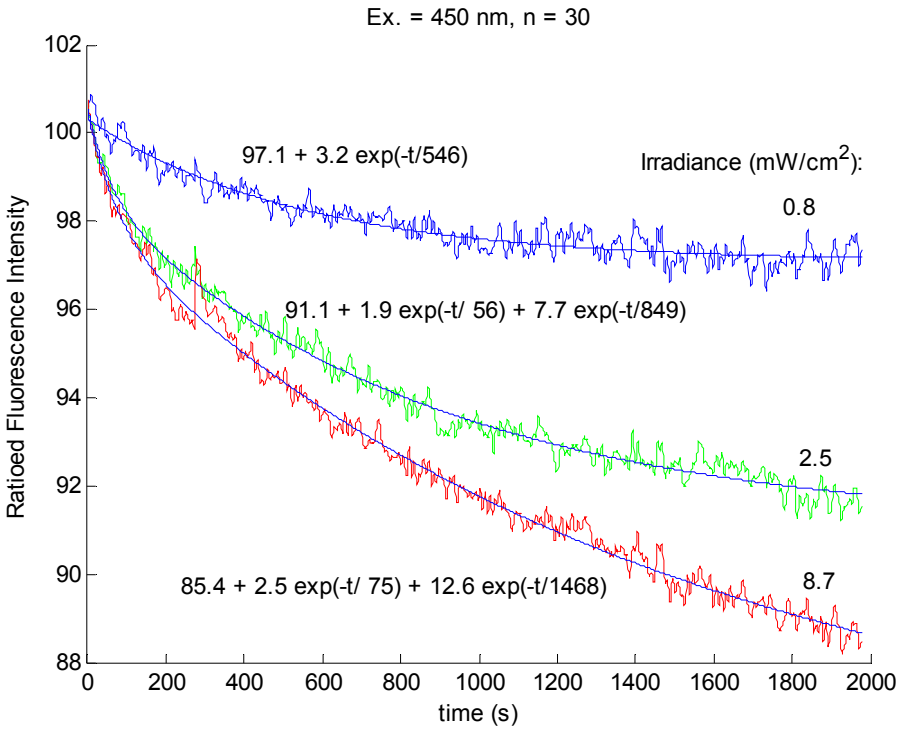

Figure 3a. Photo-decay decays averaged across all subjects. Excitation: 450 nm.

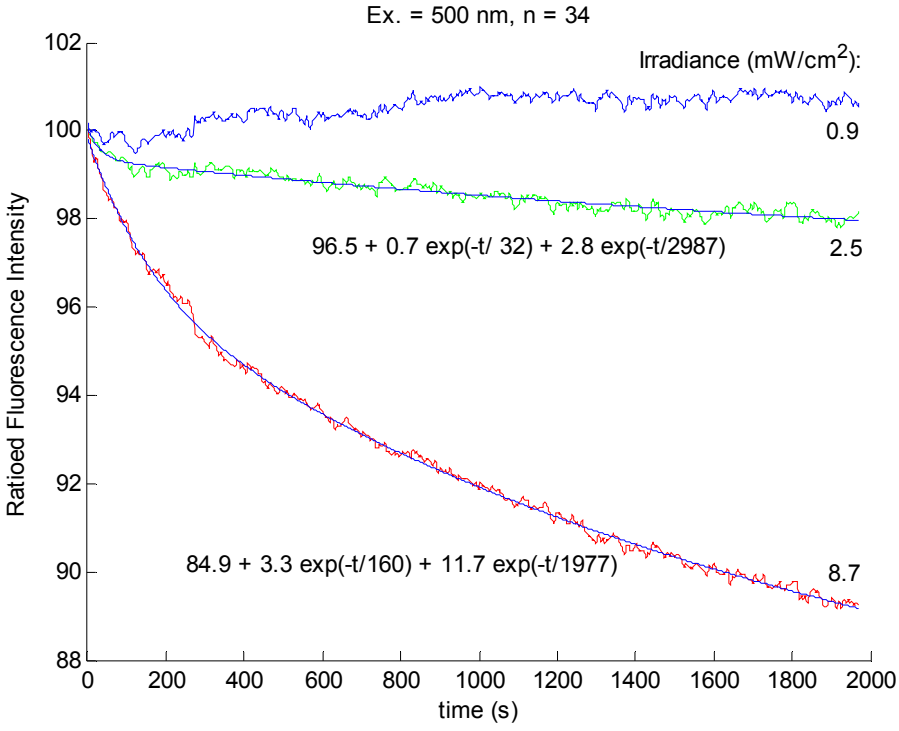

Figure 3b. Photo-decay decays averaged across all subjects. Excitation: 500 nm.

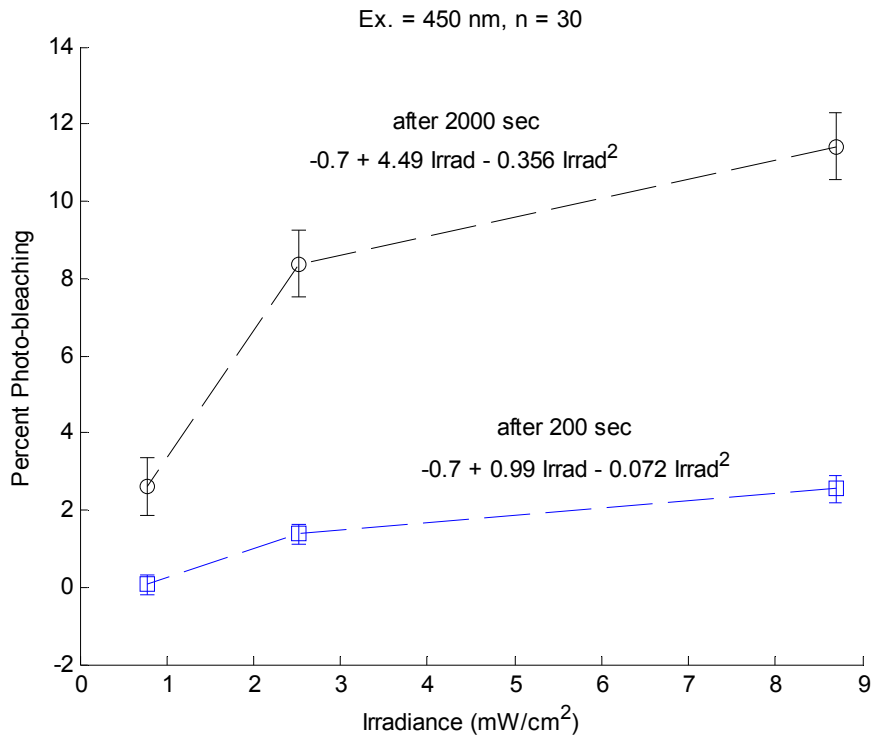

Figure 4a. Percent photo-decay as a function of irradiance. Excitation: 450 nm.

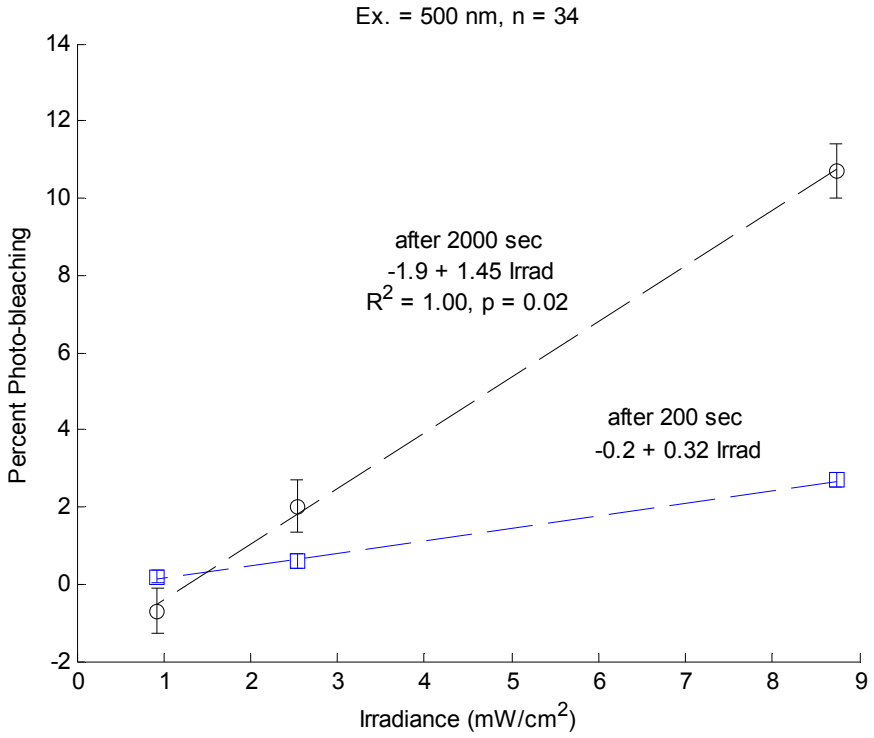

Figure 4b. Percent photo-decay as a function of irradiance. Excitation: 500 nm.

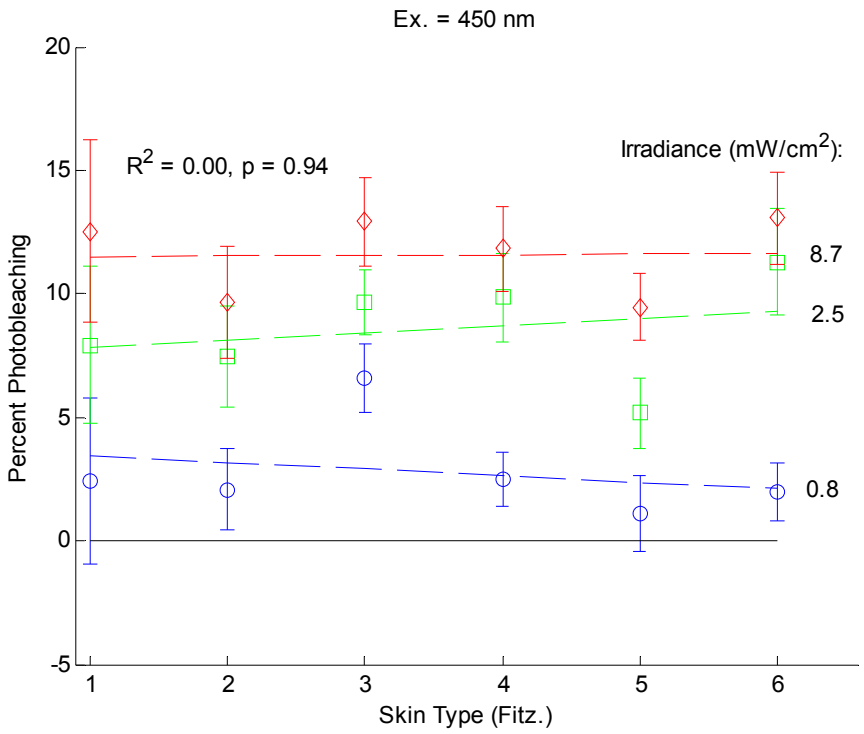

Figure 5a. Percent photo-decay as function of irradiance and skin type. Excitation: 450 nm.

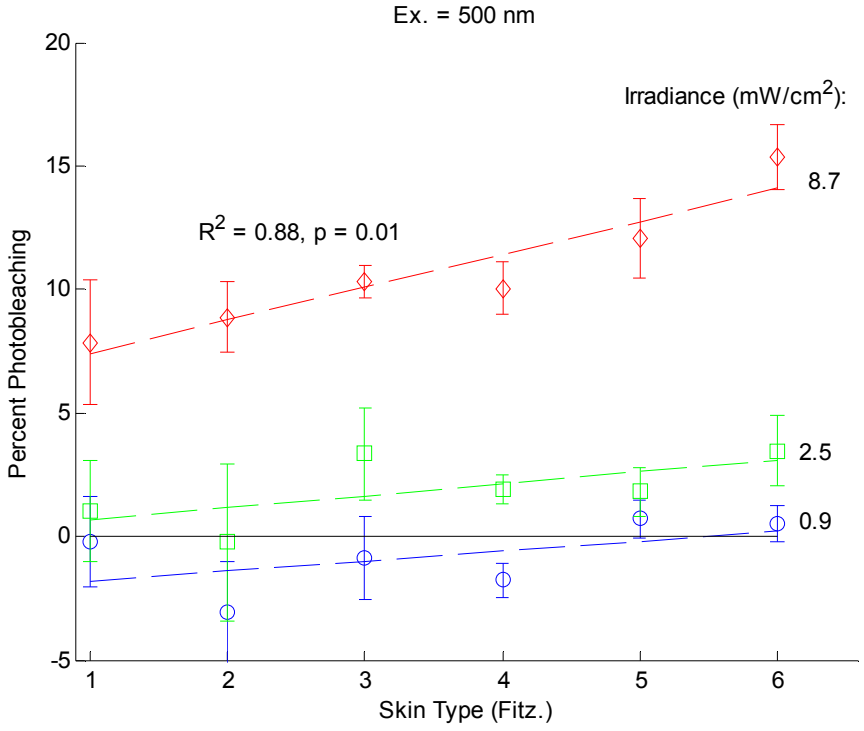

Figure 5b. Percent photo-decay as function of irradiance and skin type. Excitation: 500 nm.