RESEARCH ARTICLE

High UV Excitation Intensity Induces Photoconversion of DAPI During Wide-Field Microscopy

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Keywords:
***DAPI:** 4',6-diamidino-2-phenylindole (DAPI) is a fluorescent stain that binds strongly to A-T rich regions in DNA.*
***Photoconversion:** A photochemical alteration of chemical structure upon the absorption of electromagnetic radiation.*
***Fluorescence Microscopy:** The use of an optical microscope that measures/visualizes electromagnetic radiation emitted from a substance in response to shorter wavelength incident radiation.*
***Photobleaching:** Loss of fluorescence by a pigment when illuminated due to photochemical reactions.*

Abstract

Background: Multi-color fluorescence microscopy is dependent on the spectral specificity of the dyes and probes used for localization. One of the most commonly used fluorescent DNA dyes is DAPI, which is usually excited by UV light to emit in the blue visible light range. Herein, we describe a pattern of decreasing DAPI fluorescence upon extended UV exposure, closely followed by an increase in emission maxima in the green range.

Methods: UV-induced photo-conversion of DAPI to green-fluorescing photoproducts was studied on Chinese hamster ovary cells using wide-field fluorescence microscopy, at different UV exposure times and intensities. Imaging was done in repetitive cycles, by alternating between a DAPI and FITC filter cube and following this with a 1 second UV excitation time. The effect of differing UV light intensities on the photo-conversion process was not previously described in the literature.

Results: Upon image analysis from a large sample of cells, the rate of photo-conversion was shown to be dependent on both the duration of UV excitation and the intensity of the UV light source. Both the process of DAPI depletion and photo-product growth showed biphasic exponential patterns of change. Furthermore, the level of DAPI fluorescence intensity was found to be negatively correlated with the green fluorescence of the photo-product.

Limitations: This study did not examine the effect of differing mounting media or a variation in DAPI concentration on the rate of DAPI photo-conversion. Also, the exact light dosage to the system was not measured from the 100W Hg bulb. Photo-bleaching of green fluorescence in cells not stained with DAPI was not measured to control for bleaching of endogenous cell molecules.

Conclusion: Based on our findings, a set of recommendations was formulated in order to help reduce the effects of UV-induced DAPI photo-conversion.

Introduction

Immunocytochemistry has proven to be tremendously useful in localizing and identifying molecules of interest within cells. The fluorescent dye 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) is commonly used for visualizing the nucleus and DNA regions of the cell (1-3). DAPI binds to the minor groove of DNA strands, particularly A-T rich clusters (2, 3). Therefore, this common fluorescent dye has a multitude of applications either as a DNA-probe for flow cytometry or a dye for chromosome staining and simple DNA visualization in a variety of biochemical assays (3). Upon binding with DNA molecules, DAPI is most readily excited at 364nm wavelengths but has emission maxima in the 454nm range (4). By emitting within blue regions of the visible spectrum, DAPI nuclear staining can be used in concordance with other dyes emitting in different ranges of visible light. It is essential that the spectral properties of separate dyes remain unique and consistent, facilitating accurate fluorescence microscopy.

Besides simply exciting DAPI molecules to induce maximum fluorescence, UV light has also been shown to readily convert blue-emitting dyes such as DAPI or Hoechst 33258 into forms with green emission maxima (4-6). This UV-induced conversion generates photoproducts that not only emit light at longer wavelengths, but are also most readily excited by blue light. Through a complete spectral shift, the properties of these photo-products mimic those of fluorescein derivatives (FITC) or green fluorescent protein (GFP). If left unaccounted for, such photo-conversion could be severely detrimental to precise multi-color fluorescence microscopy due to false positive green signals in the nucleus.

Photo-conversion has also been shown to be dependent on the duration of UV-exposure yet independent of DNA binding, environment acidity/alkalinity or even the presence of a water solution (4, 6). The mechanism of this conversion is believed to be UV-induced oxidation, as studies have shown treatment of Hoechst 33258 with H_20_2 solution yielded similar increases in green intensity, although the effect was weaker with DAPI (4). Furthermore, mass spectroscopy analysis showed both UV exposure and H_20_2 increased the percentage of double protonated forms of DAPI. The photoproduct is likely the dye in this protonated state, a slight change in configuration that substantially alters the wavelengths of the excitation and emission maxima. A detailed study of DAPI can be complicated by its tendency to undergo photo-bleaching, which may or may not be a related process to photo-conversion. Likewise, the created photoproducts themselves have low stability and exhibit photo-bleaching after extended excitation. Interestingly, Zurek-Biesiada *et al.* noted that bleached DAPI signals regained ~50% of their initial levels after 1hr, while corresponding levels of the green-emitting photoproduct dropped ~50% over the same period (4). Such observations lead to speculation that the process of UV-induced photo-conversion is reversible in a subpopulation of molecules within this nuclear dye.

While planning a multi-color fluorescent assay using DAPI, it is important to consider a wide variety of factors that may potentially impact the rate of photo-conversion. Firstly, DAPI concentration has been found to be related to the rate of photo-conversion at levels between 0.1-1μM, where a higher concentration provides a larger pool of reactants to facilitate a faster conversion process (6). At high DAPI concentrations (7-10μM), fluorescent signaling is shown to decrease likely due to self-quenching, but this complication does not slow down photo-conversion (4). The increase in green intensity following UV excitation of DAPI was found when using a variety of mounting mediums and fixation protocols (5, 6). However, the use of glycerol as a mounting medium is particularly problematic as very high concentrations of glycerol in the system are proportional to the level of photo-conversion (6).

These considerations lead us to select the mounting reagent ProLong® Gold for experimentation. Despite being glycerol based, ProLong Gold reduces the extraneous impact of photo-bleaching, is a commonly used laboratory medium and would allow better comparison to previous studies which used it (4-6). Experiments using high powered lasers (e.g. 100mW and 1.25mW for excitation and imaging respectively)(4) or mercury metal halide lamps (100W)(6) have characterized DAPI photo-conversion in confocal microscopy. This study will use a metal halide lamp with a wide-field approach as done by Jez *et al.* (6)

Herein, we observe the increase of green signaling as UV exposure time increases, which will be referred to as FITC signaling due to the use of a FITC filter cube. It is the aim of this study to identify a set of protocols to reduce the rate of photo-conversion while further exploring the relationship between DAPI and its respective photoproduct. In addition, the previously unstudied effects of different UV intensities on this process were described.

Materials and Methods:

Sample preparation

Samples of Chinese hamster ovary cells expressing the focal adhesion adaptor protein Paxillin-GFP were cultured and prepared using standard protocols. The cells were stained with 100 μL of DAPI (5 mg/ml stock, 1:5000 dilution to 1 μg/ml) for 15 minutes at 23°C. They were then washed 3 times with 1ml PBS-and subsequently mounted on slides using ProLong® Gold antifade reagent (Life Technologies, Carlsbad CA, U.S).

Image acquisition

Observation of samples was done using a Zeiss AxioVert200M Inverted Microscope with motorized stage and a 63x/1.4NA oil immersion lens. Cells were first imaged using a FITC filter cube (467-498nm excitation, 513-556 emission), then using a DAPI filter cube (352-402nm excitation, 417-477nm emission) and finally were simply excited with UV light (100W Hg bulb) for a total of 1 second UV exposure per cycle. Exposure times for imaging were adjusted for optimal conditions based on the UV intensity, but total UV exposure was kept consistent. Sample cells were imaged alternately with the FITC and DAPI filter cubes for a total of 200 cycles (200s total UV exposure). Thirty different cells were imaged on 3 separate occasions for a total of 90 cells. This process was repeated for 100%, 50% and 10% intensities of the 100W mercury halide lamp for a total of 270 cells imaged.

Image analysis

Sample images were analyzed using MetaXpress 5.0 (Molecular Devices LLC, Sunnyvale CA, U.S) software. Images were first corrected for background fluorescence using the software tools and then the mean nucleic DAPI and FITC fluorescence changes was obtained for each cell. After confirming that there was no difference between the

3 sampling periods, the normalized nucleic fluorescence data of 90 cells per time-point per UV intensity were averaged together and statistical analysis was performed.

Results

Fixed and mounted Chinese hamster ovary cells with a paxillin-GFP marker were stained with DAPI. Blue and green fluorescence was observed using the DAPI and FITC filter cubes for differing UV light exposure times and intensities. It should be noted that any green signaling was referred to as "FITC signaling," but this involves green fluorescence from GFP and converted DAPI, imaged by a FITC filter cube. There is no FITC staining present, only the use of a FITC filter cube for the imaging of green fluorescence.

UV exposure causes a decrease in DAPI fluorescence

Upon exposure to UV light, there was an exponential decay in DAPI signaling probably due to both photo-bleaching and photo-conversion (Graphs 1-3, Fig.1). When comparing different UV intensities, there was a significant difference in the overall DAPI fluorescence change during the entire 200 second time course (Table 1). Among the differing intensities levels, DAPI signaling decayed most rapidly in the initial phases of UV exposure, followed by a considerably slower exponential decay component. Therefore, the following biphasic exponential decay regression was used to model this interesting trend.

$$f(x) = y_0 + a \cdot e^{-b \cdot x} + c \cdot e^{-d \cdot x}$$

The variables *b* and *d* represent the rate constants of the faster curve and slower curve respectively. All three DAPI models were well representative of the data with R^2 values of 0.9999, 0.9998 and 0.9998 for the 100%, 50% and 10% experiments and had random residual distribution (Graphs 8,10,12). The biphasic nature of this regression model is itself indicative of two separate decay process, the fast one perhaps representing photo-bleaching occurring in unison with the slower photo-conversion process.

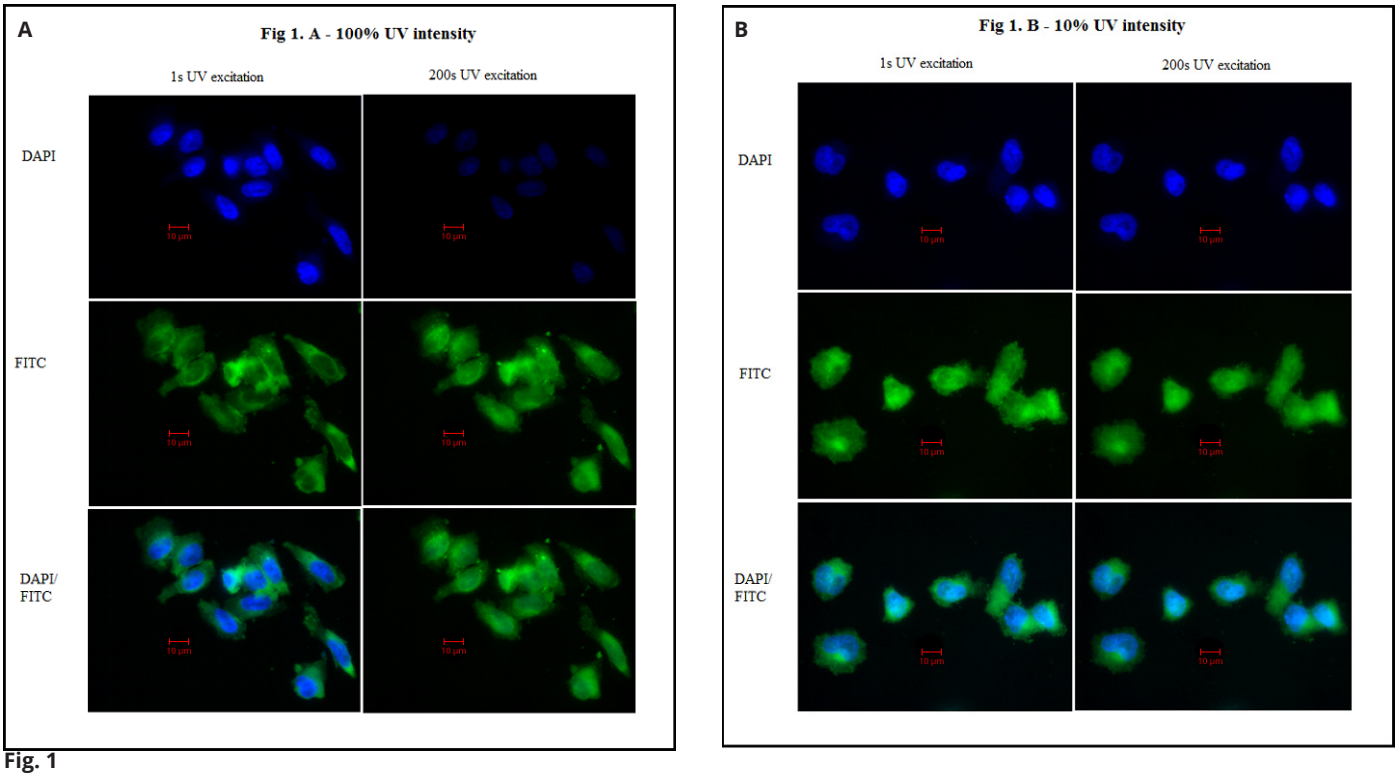


Fig. 1

Fluorescent imaging of 100% and 10% UV-induced DAPI photoconversion

A) The scale bars are 10μm in length. High DAPI fluorescence is shown in the upper left panel prior to extended 100% UV exposure. Following 200 seconds of UV excitation, the DAPI signal is almost completely extinguished while there is a corresponding increase in FITC fluorescence shown the in the middle two panels. Note that the increase in FITC fluorescence is primarily localized in the nucleus without substantial change in the cytoplasm, this supports the notion that it is the nucleic dye DAPI that is being photoconverted.

B) The scale bars are 10μm in length. The extent of DAPI depletion is not as noticeable after 200 seconds of 10% UV excitation while FITC signaling does not seem to be significantly effected also. When both the DAPI and FITC signals are overlain on top of each other, there appears to a minimal change in fluorescence due to photoconversion at this UV intensity.

	T-Test probability between different intensity groups		
Dye	100% and 50%	50% and 10%	100% and 10%
DAPI	2.11×10^{-70}	5.32×10^{-38}	9.86×10^{-93}
FITC	1.04×10^{-22}	3.42×10^{-10}	9.44×10^{-37}

Table 1. Probability associated with t-test between differing intensity groups.
Very low P-values were obtained when comparing the change in fluorescence between time points 1s and 200s, among differing intensity groups (i.e. comparing overall change in fluorescence during the time course between intensity groups). Although the differences between all three groups were significant, the greatest difference was between 100% and 10% groups, followed by the 100% and 50% groups.

Category	Fast Component			Slow Component		
UV intensity	100%	50%	10%	100%	50%	10%
Plateau (y_o)	0.083	0.218	0.365	0.083	0.218	0.365
Curve span (a or c)	0.2148	0.673	0.555	0.744	0.104	0.071
Rate constant in s^{-1} (b or d)	0.285 ± 0.032	0.147 ± 0.093	0.082 ± 0.043	0.0107 ± 0.0003	0.006 ± 0.0057	0.003 ± 0.005
Time constant (s)	3.50 ± 0.392	6.77 ± 4.28	12.1 ± 6.34	94.4 ± 2.65	158 ± 150	278 ± 463
Half-Life (s)	2.43 ± 0.273	4.69 ± 2.96	8.39 ± 4.39	64.7 ± 1.82	110 ± 104	192 ± 307

Table 2. Regression constants and half life of fast and slow DAPI curves.
The regression constants are indicated for each curve at differing UV intensity levels. 'Plateau' indicates the asymptotic value at which the curve converges after decaying over the entire curve span. The variables *a* and *b* belong to the fast component while *c* and *d* are from the slow component. The time constant is the inverse of the rate constant value, while the half life was calculated as ln2/(rate constant). The standard deviation values of the rate constant, time constant and half life are based on three separate trial curves obtained for each intensity experiment.

The three separate UV intensities differed substantially in the rate, at which DAPI signaling decreased, with the fastest rates present at higher UV intensities. The regression models predicted different plateaus for each of the three intensities. The plateau value for 100% UV intensity as a fraction was 0.08 of the original standardized maximal value, 0.22 for 50% UV intensity and finally 0.37 for 10% UV intensity (Table 2). Although these differences may be attributed to inaccuracies in the model, they suggest that higher UV intensity achieves more complete depletion of the DAPI pool. There also appear to be inherent threshold values determining the extent of DAPI depletion using the given apparatus as the model predicts 8% of the pool will always remain in theory, while closer to 18% remained in the 200 seconds actually tested (Table 2).

Comparatively, the fast components had a smaller span (distance between the plateau and starting point) than their faster counterparts for all UV intensities. Since the plateau is dependent on the span of the two separate components, the component with the greater span has a greater overall impact on how high the plateau of the regression will be. Despite having a slower rate constant, the slower process (photo-conversion) may have a larger final impact on DAPI depletion than photo-bleaching since this component has a larger 'span.' It is likely that the fast component has a reduced significance after the initial phases of UV exposure. Likewise, the rate constants of the fast components were over an order of magnitude greater than those of the slower components for all intensities. Among the fast/slow components themselves, the rate constants became smaller as the UV intensities decreased. This is subsequently reflected in the half-lives of the experiments, where there is a doubling in half-life between 100% UV intensity and 50%, followed by another doubling between 50% and 10%. This asymmetric trend in doubling indicates that a decrease in DAPI depletion is not related to UV intensity in a linear fashion, rather lower UV intensities have less of a decrease in depletion rate. In this respect, both the fast curves and slow curves mirrored each other and UV intensity seems to affect both processes equally. The standard deviation for the rate constant and half-life were based on three separate trial curves made per experiment. The standard deviations between these three trial regressions were greatest in the 50% and 10% UV intensities, particularly for the fast components.

The standard deviation of mean DAPI fluorescence was based on 90 samples for each intensity (all three trials averaged together) and gradually increased as UV exposure time went up. Although this increase in variation is to be expected in normalized data where nearly

all samples were initially at 100% fluorescence prior to UV exposure, it is indicative of differing rates of DAPI depletion among cells. These differing rates could be attributed to the differing individual contributions of photo-bleaching and photo-conversion within each sampled cell. The standard deviation was the highest at 10% UV intensity, it is likely that this UV level does not meet the threshold needed to induce a change in DAPI fluorescence in certain cells, thus causing more variability in the data than at high UV levels.

UV exposure causes an increase in FITC/GFP fluorescence

Concordant with an apparent decrease in DAPI fluorescence was an increase in the strength of FITC signaling that was reflective of exponential association (Fig. 1, Graphs 4-6). Once again, the data was noted to express two separate phases while increasing, therefore the following biphasic regression model was used:

$$f(x) = y_0 + a\cdot(1 - e - b\cdot x) + c\cdot(1 - e - d\cdot x)$$

The regression curves generated R^2 values of 0.9999, 0.9998 and 0.9977 for the 100%, 50% and 10% UV intensities respectively, while the residual values were low and random if the initial outlier values were excluded (Graphs 7,9,11). These outlier values present during the first few seconds of UV exposure were excluded from curve modeling (indicated by black dots in Graphs 4-6). Apart from possessing high variability and large standard deviation values, the outlier values were exceptions to the general increasing trend as they indicated rapid drops in fluorescence within the initial phases of UV excitation. The drop in fluorescence may be explained as initial photo-bleaching of the control levels of green fluorescence already within the cell prior to conversion (paxillin-GFP label), where this initial drop is quickly overcompensated for by the slower but more substantial DAPI photo-conversion process. In addition, the biphasic nature of the regression independent of these outliers could point to both a fast and slow conversion processes for increasing FITC fluorescence.

The vast majority of the 200 time points were fitted to exponential association curves, with initial starting points of 0.44, 0.63 and 0.80 for the 100%, 50% and 10% UV intensities (Table 3). A lower starting point from the maximal normalized value of 1 (often reached near the last time point) indicated a greater overall percentage of conversion. Although the general trend of increasing FITC signal strength was apparent at 100% UV intensity and to a lesser extent in the 50% UV data, the initial drop attributed to FITC photo-bleaching often canceled out photo-conversion at 10% so that FITC fluorescence levels would simply reach their starting point at the end of 200 seconds in many cells. The large standard deviation during these initial time points reflected a wide range of spectral behaviors among cells, where some did not exhibit bleaching phenotypes while others had a massive drop in FITC fluorescence that was only later followed by a conversion increase.

The slow components showed the same trend described in the DAPI models where there was a doubling in half time between 100% and 50%, followed by another doubling between 50% and 10%. In contrast, the fast FITC components showed a half-time doubling between 100% and 50% UV intensities, but a 25-fold increase between 50% and 10%. Thus, instead of there being an order of magnitude difference between the rate constants of the fast and slow components, they were the exact same at 10% UV intensity. This may indicate that the slow process increasing FITC fluorescence follows an expected trend at 10% UV, while the faster component is substantially inhibited from what is expected and may be the reason for an inhibited photo-conversion process. Additionally, it was half lives of the 10% fast and slow components that displayed the greatest standard deviation, which was calculated between the three separate trial curves for each experiment. Thus, there was substantial variation in the rate constants even among individual 30 cell trials, where 10% UV strength was not enough to induce a unanimous photo-conversion response.

Category	Fast Component			Slow Component		
UV intensity	100%	50%	10%	100%	50%	10%
Initial point (y_o)	0.443	0.635	0.801	0.443	0.635	0.801
Curve span (a or c)	0.211	0.094	0.382	0.386	0.393	0.375
Rate constant in s^{-1} (b or d)	0.080 ± 0.008	0.038 ± 0.014	0.002 ± 0.002	0.011 ± 0.001	0.005 ± 0.0005	0.002 ± 0.002
Time constant (s)	12.5 ± 1.20	26.0 ± 9.58	666 ± 666	94.3 ± 8.57	185 ± 18.5	666 ± 666
Half-Life (s)	8.63 ± 0.863	18.1 ± 6.67	462 ± 462	65.4 ± 5.94	128 ±1 2.8	462 ± 462

Table 3. Regression constants and half time of fast and slow FITC curves.
The regression constants are indicated for each curve at differing UV intensity levels. 'Initial point' indicates the value at which the curve meets the y-axis at 0 seconds. The variables a and b belong to the fast component while c and d are from the slow component. The time constant is the inverse of the rate constant value, while the half time was calculated as ln2/(rate constant). The standard deviation values of the rate constant, time constant and half life are based on three separate trial curves obtained for each intensity experiment.

Negative correlation between DAPI and FITC fluorescence

To better evaluate whether the decreases in DAPI signal strength were more attributable to photo-bleaching or photo-conversion, the level of both DAPI and FITC fluorescence were compared at equal UV exposure time points with linear regressions (R^2 values of 0.99). For all three intensities, a direct negative correlation was apparent between DAPI and FITC fluorescence (Graph 13). The ratio of DAPI:FITC fluorescence was not 1:1, instead the slope was -1.7, -1.5 and -1.4 for the 100%, 50% and 10% UV intensities respectively. Thus, there is a greater drop in DAPI fluorescence than increase in FITC fluorescence. It can therefore be concluded that the UV-induced decrease in DAPI signal could mostly be attributed to photo-conversion to green-emitting photoproducts. Photo-bleaching still does have a significant impact on DAPI depletion despite the use of ProLong Gold and its effect was greater at higher intensities than lower ones (more negative slope). Cells exposed to lower UV intensities did have higher DAPI to FITC ratio starting points compared to their counterparts samples exposed to higher UV intensities.

Additionally, outside of each linear regression there was a tail pattern that became more apparent at lower UV intensities. FITC signaling quickly dropped at high-DAPI/low-FITC levels (corresponding to early UV exposure time-points). This peculiarity could once again be attributed to early photo-bleaching of the control FITC fluorescence in the cell prior to conversion, where the green emitting molecules are bleaching faster than can be compensated by early photo-conversion.

Discussion

The depletion of DAPI in response to UV excitation has been demonstrated to be directly related to an increase in FITC fluorescence and thereby an increase in green-emitting DAPI photoproducts. At all intensities, the duration of UV exposure was related to the level of photo-conversion. Lower conversion rates and partial saturation of the system occurred at around 100 seconds at 100% UV intensity. Previous studies have observed a conversion plateau between 32 seconds and 4 minutes, probably attributable to the initial size of the conversion reactant pool (DAPI concentration) and the power of the UV light source (4-6). We used a similar set-up to Jez *et al*, (100W metal halide lamp/wide-field) who found a plateau of 32 seconds at 100% UV intensity. However, this was done using a mounting medium with a high glycerol concentration, while using ProLong Gold they too described the response as significantly less robust (6). Although the automated intermittent FITC imaging during UV excitation may have slowed down the rate of DAPI photo-conversion by giving time for a reverse reaction, this is unlikely significant as reversal occurs on the order of minutes to hours and not fractions of a second (4).

DAPI fluorescence decayed in a biphasic exponential manner, likely due to the initial fast process of photo-bleaching followed by slower photo-conversion. The reason that photo-bleaching was hypothesized to be the slower process is due to the lagging increase in the FITC signal behind the initial DAPI drop. The green-emitting molecules already present in the cells experienced their own photo-bleaching effect, but this was short lived during early UV exposure times and quickly compensated for by an increase in photo-conversion products. The photoproducts themselves also showed a biphasic pattern of growth, ultimately leveling off as DAPI substrate concentrations decreased. One hypothesis for the two-phase nature of this growth could be that the direct and fast process of DAPI-to-photoproduct conversion occurs alongside a slower indirect process of bleached DAPI-to-photoproduct conversion. As the initial pool of pure DAPI disappears, the growth curve shifts more and more to this secondary slower process that requires additional steps before the substrate can indirectly be converted to the photoproduct. An alternative mechanism would involve an increase in the rate of the reverse reaction, alongside a decrease in the availability of DAPI substrate as the photoproduct concentration increases. These two factors would gradually shift the equilibrium towards a slower increase and potentially reduce the intensity of FITC signaling completely below the sensitivity of the assay if observed long enough.

The rate of photo-conversion was substantially lower while using 10% UV concentrations, where most of the conversion inhibition seemed to originate from an inhibition of the fast component rate constant. Instead of being an order of magnitude faster than the slow component as was the case with the other intensities, the 10% fast component has the same half-life as the slow component. It is possible that 10% UV intensity is below a given threshold needed to sufficiently allow the fast process to overcome the initial dip in FITC fluorescence due to bleaching of green-emitting molecules. This study did not examine the effect of differing mounting media or a variation in DAPI concentration on the rate of DAPI photo-conversion. Also, the exact light dosage to the system was not measured from the 100W Hg bulb. Photo-bleaching of green fluorescence in cells not stained with DAPI was not measured, thus the speculated photo-bleaching quickly occurring at the beginning of excitation was not controlled for. Instead of looking at the change in green fluorescence in cells labelled with Paxillin-GFP, using a cell line with no endogenous green fluorescence and a red counterstain could have avoided the problem of inherent green photo-bleaching within the cell's endogenous molecules.

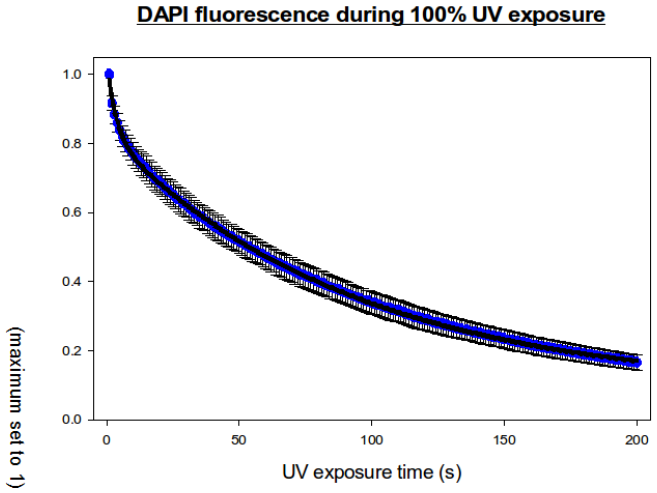
Conclusions

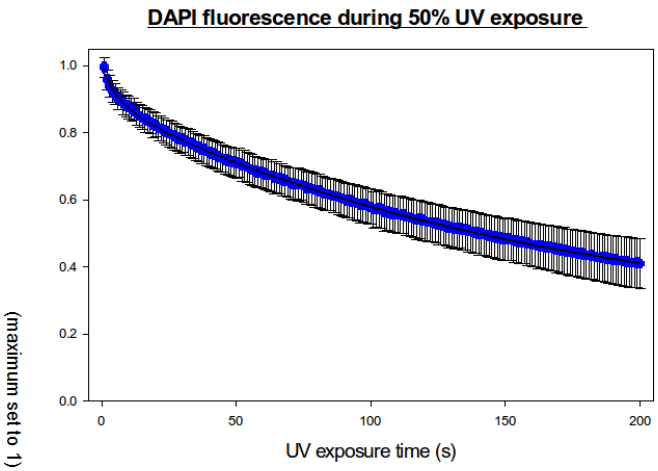
Based on these findings, the following set of protocols were formulated in order to reduce the potential negative consequences of unexpected DAPI photo-conversion:

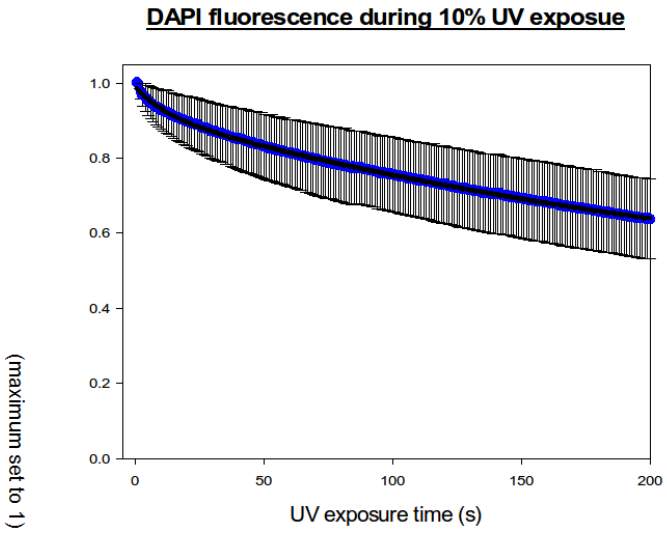
1. UV intensities should be kept at 10% that of standard levels (100% intensity of a 100W Hg bulb) or less whenever possible, despite the needed increase in imaging exposure times.
2. Limit total UV exposure times to a few seconds at 100% UV intensity and up to 50 seconds at 10% intensity (at which point FITC fluorescence returns to control levels after dipping initially).
3. The use of lowest DAPI staining concentrations possible and mounting agents with lower glycerol levels (6).
4. Acquire DAPI images after all other higher wavelength images in order to eliminate green emission false positives.
5. Use alternate DNA dyes such as DRAQ5 which fluoresce in the far red regions of the spectrum and has not been shown to exhibit photo-conversion (7). Hoechst 3258 and Vybrant DyeCycle blue dyes are not suitable alternatives as they experience photo-conversion at a comparable level to DAPI (4).

Acknowledgments

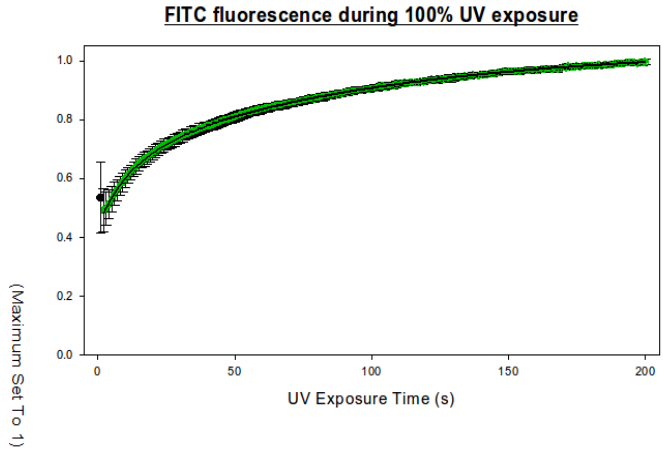
A special thanks to the tireless help and guidance of Dr. Claire Brown and Erika Wee throughout this project. Their expertise and kindness was invaluable for the completion of this study.

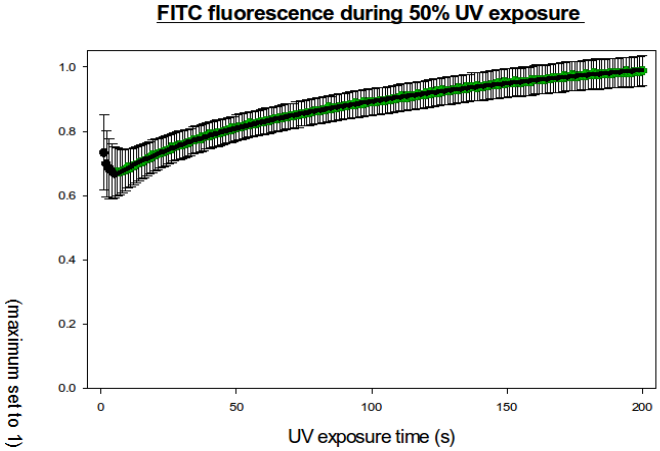


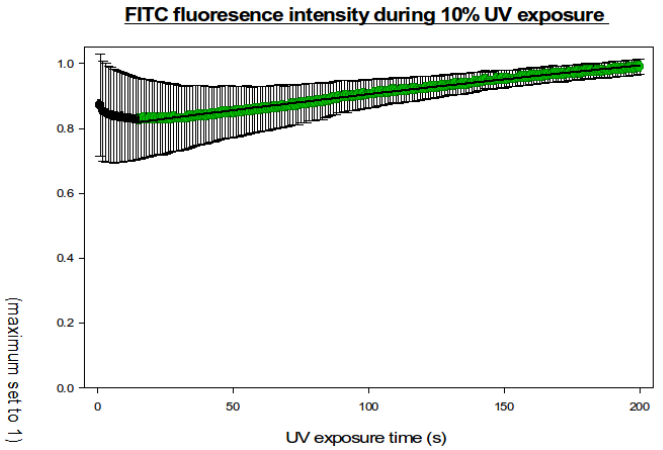




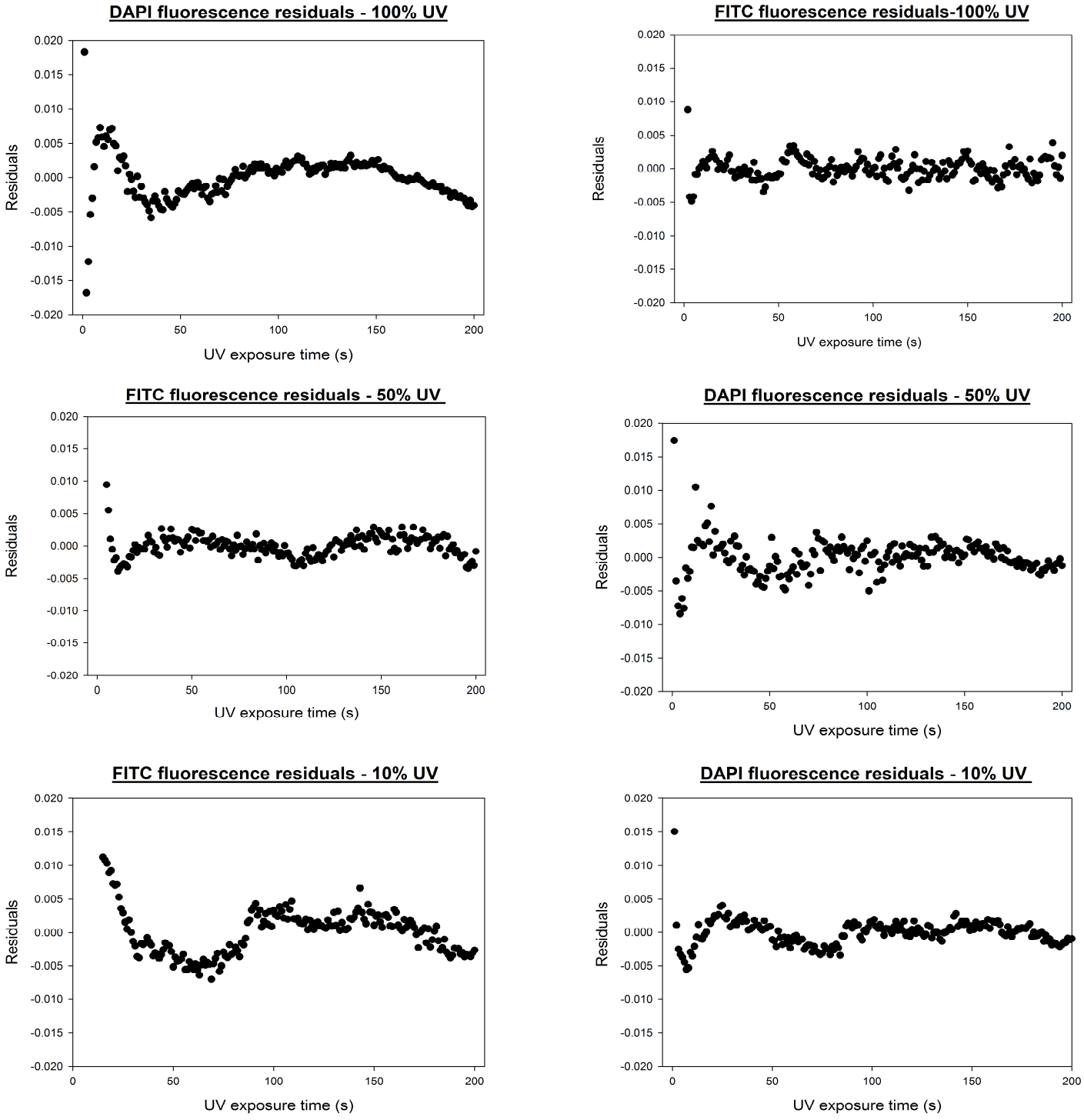
Graph 1-3. DAPI fluorescence during UV exposure. **Graph 1** shows a substantial decrease in normalized DAPI fluorescence after 200 seconds of 100% UV excitation. This trend is not as apparent in **Graph 2** and is even weaker in **Graph 3** at 10% UV exposure levels, where there was large variability in fluorescence at individual time points as indicated by the large error bars representing the fluorescence standard deviation. When the UV intensity is high, most cells follow expeirence biphasic exponential DAPI signal decay, however at lower UV levels many cells do not experience this effect uniformly.





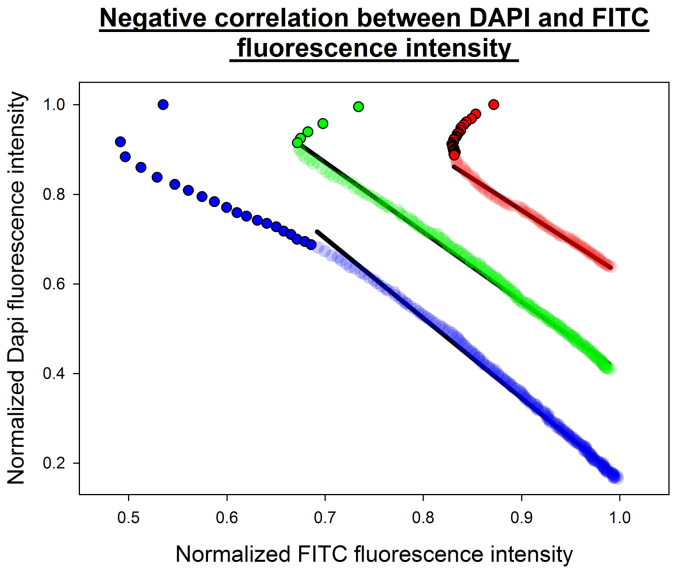


Graph 4-6. FITC fluorescence during UV exposure. **Graph 4** shows a substantial increase in normalized fluorescence of FITC after 200 seconds of 100% UV excitation. This exponential biphasic trend is only interruppted by variable outliers in the initial phase of excitation, represented by black dots with large error bars. The exponential association is not as apparent in **Graph 5** and is even weaker in **Graph 6** at 10% UV exposure levels, where there was large variability in fluorescence at individual time points as indicated by the error bars



Graph 7-12. Regression residuals.

The residuals of Graphs 1-6 are shown individually to assess the quality of the exponential regression used for modeling. All residual data indicates a random distribution alongside relatively insignificant distances between the predicted values and the actual experimental values, indicating the regressions are a good fit. It should be noted that the initial decreasing outliers for the previous FITC graphs were not included as part of the regressions and therefore do not have representative residual values.



Graph 13. Negative correlation between DAPI and FITC fluorescent intensity.
The right, middle and left circles indicate 10%, 50% and 100% UV intensity time point values respectively. The transparent datapoints represent those included in the linear regression formula. The solid color datapoints are outliers to this linear correlation trend Linear regression are indicated by black lines, with slopes of -1.78, -1.56 and 1.41 for the 100%, 50% and 10% UV intensities.

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