Optimization of fluorescent imaging in the operating room through pulsed acquisition and gating to ambient background cycling

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Abstract: The design of fluorescence imaging instruments for surgical guidance is rapidly evolving, and a key issue is to efficiently capture signals with high ambient room lighting. Here, we introduce a novel time-gated approach to fluorescence imaging synchronizing acquisition to the 120 Hz light of the room, with pulsed LED excitation and gated ICCD detection. It is shown that under bright ambient room light this technique allows for the detection of physiologically relevant nanomolar fluorophore concentrations, and in particular reduces the light fluctuations present from the room lights, making low concentration measurements more reliable. This is particularly relevant for the light bands near 700nm that are more dominated by ambient lights.

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OCIS codes: (170.3890) Medical optics instrumentation; (170.2945) Illumination design.

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1. Introduction

The potential role for fluorescence imaging during surgery to help guide resection has been investigated intensely in recent years [1–4], with many commercial systems coming onto the market. Fluorescence cannot only be used to mark sites of pathology for resection but also to mark sensitive areas for the surgeon to avoid [5–9]. Increased availability of specific fluorescent probes which delineate ducts, tissues and/or molecular expression will continue, and these will gradually find successful applications in surgical procedures. However, one of the greatest hindrances to the adoption of fluorescence guided surgery (FGS) is in the change to surgical workflow currently required to visualize fluorescence. This study examines a major requirement affecting clinical workflow; the need to reject the temporal cycling of background room light signal during fluorescence imaging.

For most fluorescent imaging applications, it is critical to maximize fluorescence signal relative to non-specific background light signals [10–14]. Current clinical methods to remove background ambient light from signal require either the room lights to be shut off as is the case in 5-ALA induced PpIX imaging [15] or the use of wavelength filtering as is the case in indocyanine green (ICG) imaging [16, 17]. 5-ALA induced PpIX imaging for glioma resection is one of the most promising applications of fluorescence in surgical guidance [18]. While this technique has been used in a number of clinical trials and is currently the standard of care in Germany [18] the current methods of fluorescence visualization require that all background lights in the operating room be turned off. Room lights can be turned off intermittently during surgery for fluorescence imaging, however, this method is a major disruption to workflow, and a potential hazard which will ultimately limit widespread adoption. This is particularly important for visible light fluorescence, because the wavelength filtering is less effective when high ambient background light is in the same bandwidth as the fluorescence to be detected.
Most fluorescence imaging today is done with ICG, for vascular identification and flow imaging. For this tracer, the emission band is in the near infrared (NIR), facilitating the use of optical filtering since most OR’s have relatively low ambient NIR intensity [12] and ICG can be repeatedly administered at high doses (millimolar), and so the majority of the detected NIR signal from background lights is relatively low in comparison to high ICG emissions. This can work well for ICG, however, as these systems are used for fluorescence imaging with molecular reporters which are injected at much lower concentrations (nanomolar to micromolar), it is likely that the detected emission signals may be 3 to 6 orders of magnitude lower than the ambient NIR signal, and so additional methods to remove the background will be needed.

A pulsed imaging system was previously developed [19] to image fluorescence in fast snapshots, enabling real-time background subtraction as well the maximization of fluorescence to background signal. The idea was to enable fluorescence imaging under normal surgical background lighting even when utilizing visible light fluorescence or imaging extremely low fluorophore concentrations. The system uses pulsed light from LEDs and a gated-intensified CCD camera for acquisition. One major benefit of this gated system is that when LEDs are pulsed at low duty cycle, they can be over-driven with a higher current, leading to very high irradiance which increases fluorescence to background ratios. The additional benefit of the design is that the intensified CCD captures signals with an amplified gain of several orders of magnitude, thereby maximizing the fluorophore sensitivity even at these low integration times. This system was used in an earlier study where it was shown this method of pulsed light imaging was able to reduce the background light contribution to the recorded signal and enable PpIX fluorescence imaging under normal ambient light (~35 µW/cm² provided by a series of Sylvania Octron XP 17W 3500K fluorescent overhead room lights) [19].

However, further testing showed that fluorescence signals for physiologically relevant concentrations of both IRDye 680RD and PpIX become overwhelmed by background light signal in the brighter environments common in the OR. A large background signal does not necessarily prohibit effective fluorescence imaging, but fluctuations in background signal that are on the order of the fluorescence signal transiently interfere with the lower signals in a non-repeateable way. The problem is avoided if acquisition times are long enough that the periodic nature of the background signal averages out. However, a frequency of 120Hz translates to a period of 8.3ms, which means that this will not be the case for sub-millisecond acquisition times. It was hypothesized here that these fluctuations could be eliminated, regardless of acquisition time, by synchronizing the gated acquisition to the frequency of the background light. Additionally, actual background intensity can be minimized by timing acquisition to occur when background light signal reaches its lowest point. This technique has the potential to enable pulsed light FGS imaging without any alteration to standard OR lighting.

This method enables minimization of room light signal contribution as well as a drastic reduction in signal noise with fluctuations in background due to the alternating current drivers eliminated. The end goal is the ability to perform video rate imaging of physiologically relevant molecular fluorophore concentrations, at both the 700 nm and 800 nm channels under the intense ambient light conditions of a typical OR. As was done previously, images are acquired with and without the excitation light to allow further suppression of the background ambient light through presentation of subtracted images. This subtraction imaging is very efficient as long as the variation in background ambient light is only a small fraction of the fluorescence signal. The gating methodology presented here was proposed to suppress variations in ambient background signals as well as background signal intensity to enable high fluorescence sensitivity under normal OR light conditions. At the same time this method minimizes image acquisition times, which not only increases refresh rates, but also enables LED overdriving further increasing fluorescence to background ratios.
2. Materials and methods

2.1 Ambient room light measurements

In order to characterize the background light signals typically found in an OR with high powered fluorescent overhead lights, a number of measurements were taken under these conditions. A power meter (PM100, ThorLabs) was used to estimate the average continuous surface irradiance in two separate surgical rooms, both illuminated with a series of overhead fluorescent bulbs (Sylvania FO32/735/ECO). Temporal measurements were also taken at a sample rate of 48 kHz using a photodiode (DET10A Si Based Detector 200-1100 nm, ThorLabs) and data acquisition board (NI DAQ 6009). The emission spectrum of lights in each room was also acquired using a compact spectrometer (QE 65000, Ocean Optics). The relative magnitude of these was scaled to the average power measured with the power meter. All measurements were taken at the center of the operating room, at a height of approximately 100 cm and in full view of all overhead fluorescent lights. This location was chosen to best simulate approximate patient location. All surgical and other light sources were off for these measurements and window shades were closed.

2.2 Pulsed fluorescence imaging system

Photographs of the pulsed imaging system are presented in Fig. 1. The bulk of the technical details of this system are described in a previous publication, and so only a brief description including updates to the system will be provided here [19]. The system is composed of two separate imaging channels, a 700 nm channel capable of imaging both PpIX and IRDye 680RD as well as an 800 nm channel capable of imaging IRDye 800CW. The 700 nm channel utilizes four 630 nm SpecBright LEDs (ProPhotonix, Cork, Ireland) with 1.0 in. diameter, 650 nm short pass filters (Edmund Optics, Barrington, NJ). The 800 nm channel, which has been added since the previous publication utilizes four 740 nm SpecBright LEDs (ProPhotonix, Cork, Ireland) with 1.0 in. diameter, 750 SP filters (Edmund Optics, Barrington, NJ). On the emission side the 700 nm channel utilizes a 700/40BP interference filter (Omega, Brattleboro, Vermont) while the 800 nm channel uses an 800/40BP interference filter (Omega, Brattleboro, Vermont) as well as a 780 nm LP absorption filter (FGL 780, ThorLabs, Newton, NJ) placed behind the interference filter. The increased excitation power due to LED overdriving makes proper filtering critical to a system such as this. Significant bleed through from higher angle light away from the center of the field of view (FOV) necessitated the use of the absorption filter on the emission side in the 800 nm channel. This solution proved far more effective than the use of a reduced aperture in combating the inefficiency of the interference filter at higher incidence angles [20].

The system utilizes the PI-MAX 3-1024 x 256 camera (26 μm pixel size) (Princeton Instr. Acton MA) in combination with a 70 mm, f2.8 lens (Sigma, Ronkonkoma NY). At a working distance of approximately 18 cm this configuration provides a maximum square field of view of approximately 1.6 cm by 1.6 cm with a spatial resolution of approximately 100 μm as determined using a standard three-bar resolving power test target (USAF-1951, NT53-714, Edmund Optics) [20].
The pulsed system was configured to allow triggering directly from an input signal provided by the background room lights. A photodiode detector (DET10A Si Based Detector 200-1100 nm, ThorLabs) was positioned to monitor background room lights, coupled to an operational amplifier (Op Amp, Texas Instruments, LM741) to amplify the signal to be sampled by a DAQ board (NI USB-6351, X Series DAQ) at 10 kHz rate. The DAQ board was used to output a trigger signal at a predefined point in the characteristic 120 Hz cycle (double the electric power line frequency) of the background signal level. The signal level used for triggering was adjusted to time image acquisition to the background signal minimum. As previously discussed, the use of short (sub millisecond) acquisition times allows for LED over-driving and provided the duty cycle is low enough enables the instantaneous driving current to be considerably higher than is possible under continuous current. The LEDs used with this system (SpecBright 630 nm and 740 nm Area Lights) are able to provide 10X higher power in pulsed mode than when they are driven in continuous wave (CW) mode (provided pulse widths are below 1ms and the duty cycle is below 5%). In these studies, 10X over driving was used with both sets of LEDs in order to maximize fluorescence signal relative to background signal. It should be noted that overdriving of LEDs will significantly shorten their useful lifetime.

3. Results

3.1 Ambient light characteristics

The temporal power variations for two sampled operating rooms are shown in Fig. 2(a), with average powers readings of 182 and 124 µW/cm². Temporal measurements in both ORs displayed strong periodic fluctuations at 120Hz.

The measured spectrum of fluorescence lighting in a typical OR is shown in Fig. 2(b) and Fig. 2(c). It can be seen that there is substantially greater signal in the wavelength band of 700 nm than in the vicinity of 800 nm. Readings taken in surgical room 1 using the power meter, when the sensor was covered with a 700/40BP filter (Omega) and then an 800/40BP filter (Omega) produced measurements of 5.0 and 0.4 µW/cm², respectively. This amounts to an approximately 12-fold greater background signal at the 700 nm channel than at the 800 nm channel.
Fig. 2. Characteristics of the ambient light signal in an Operating Room (Sylvania FO32/735/ECO overhead fluorescent lights) are shown, with (a) the temporal signals from two different rooms, showing the periodic signal at 120 Hz. The optical spectrum recorded is displayed on a linear scale (b) and logarithmic scale (c). The signal at 700 nm (visible) can be seen to be substantially larger than that at 800 nm (NIR).

3.2 Tissue phantom testing: gated acquisition with and without room light based triggering at 700 nm

Fluorescence detection levels in the OR (surgical room 1) were tested using liquid tissue simulating phantoms. Serial dilutions of IRDye 680RD (LICOR Biosciences, Lincoln, NE)) (1% Intralipid and 0.01% India ink for absorption and reduced scattering coefficients of $\mu_a = 0.02 \text{cm}^{-1}$ and $\mu_s' = 24 \text{cm}^{-1}$ respectively) were examined under a variety of acquisition settings. Liquid phantoms were contained in 1.25 cm deep, 1.6 cm square wells machined in black Delran (McMaster-Carr, Robbinsville, NJ). All reported signals were taken from 3 mm wide square regions of interest in the center of the wells (2600 pixels).

The standard method that had been used previously to successfully image both IRDye 680RD and PpIX under lower intensity lab lighting conditions was unsuccessful in the OR due to much higher background. This previous method used 1 ms gate widths, 10 X overdriving of LEDs, full camera gain and background subtraction. With acquisition times of only 1 ms, strong background light signal and large signal fluctuations at 120 Hz, fluorescence signals are overwhelmed by variations in detected background signal. This can be clearly seen in Fig. 3(a) where a series of 50 images was taken at each fluorophore concentration and the error bar plots are displayed. Error bar plots for the background subtracted signals indicate that the variation in each point is so high that differentiation between signals based on single images would not possible even at the highest concentration examined (3.9 nM). The implementation of room light based triggering was able to alleviate this problem, as shown in Fig. 3(b), where the same acquisition settings were used but this time triggering was either based on room light signals (offset right/blue) or imaging was done in complete darkness (offset right/black). The reduction in signal variation is drastic and the background subtracted signals using room light based triggering are very similar to those seen when imaging in a completely darkened room. The percent standard deviation in background subtracted signal for the scenario without room light based triggering ranges from over 150% at some of the lowest concentrations to a minimum of 24% at the highest concentration (3.9 nM). The percent standard deviation when room light based triggering is used never goes above 2% and stays below 0.5% at the highest concentration. This variation is much more in line with what is seen in the completely dark room where percent standard deviation ranges from just below 1% at the lowest concentrations to around 0.3% at the highest concentration.
Fig. 3. Fluorescence detected in the OR without (a) and with (b) room light based triggering is shown. Error bar plots showing mean and one standard deviation above and below the mean for background subtracted signals averaged from 50 repeated images of 0 to 4 nM IRDye 680RD. Acquisitions utilized 1ms gate widths, full camera gain and 10x overdriving of the LEDs. Also in (b) images were taken with room light based triggering (offset left / blue) and images taken in complete darkness (offset right / black).

In order to provide a more visual illustration of these results, actual background subtracted phantom images are presented in Fig. 4. Phantom images over the range of concentrations are shown. These are single representative images taken from the series of images used to provide the data displayed in Fig. 3.

Fig. 4. Single fluorescence background subtracted images of approximately 1.6cm by 1.6cm phantoms taken with 1ms gate widths and full camera gain. Images were taken separately and have been stitched together for viewing purposes. (a) Images taken without room light based triggering clearly demonstrate the inability to visualize fluorescence with strong variations in signal due to room light fluctuation. In (b) images taken with room light based triggering demonstrate significant fluorescence visualization at the higher concentrations examined. In (c) images taken in the absence of room lights show comparable fluorescence visualization to those seen in (b).

Figure 5(a) allows comparison of the average fluorescence and background signals at 0.25 nM IRDye 680RD phantom (averaged over 50 images) both with and without room light based triggering as well in a darkened room. It was seen that while room light based triggering reduces the contribution from background light by approximately 35%, background signal still constitutes the majority of the detected signal as compared to the darkened room where it is only a small fraction (less than 7% and known to be both nearly constant and uniform across the field). Figure 5(b) shows the much larger portion of signal that comes from background as compared to fluorescence when longer exposure times are used, LEDs are not overdriven and camera gain is not used.
The inability to perform pulsed light imaging of nanoMolar range concentrations under the OR lighting conditions described here, without the use of room light based triggering, is a consequence of using acquisition times that are only one eighth the period of the room light signal.

The potential of fluorescent imaging under the described lighting conditions using longer acquisition times was examined with acquisition times of 10, 20, 40, 100 and 200 ms and can be seen in Fig. 6. The effect of acquisition time on background signal variation can be seen in Fig. 6(a) where the standard deviations from 30 to 50 background images using various acquisition settings are divided by the corresponding average signal and compared. The drastic reduction in variation resulting from room light based triggering is easily observable as are the reductions in variation with gate width increasing from 1 ms all the way out to 200 ms. However, the quality of background subtracted fluorescence images is more a function of the level of background variation in relation to the fluorescence portion of the signal. Figure 6(b) shows the standard deviation from background images divided by the fluorescence portion of signal for the 0.25 nM IRDye 680RD phantom. Here it can be seen that despite the reduction in background signal variation from longer acquisition times, these longer acquisition times also see a lower fraction of signal from fluorescence (see Fig. 5 for a comparison of background to fluorescence signals) and as a result, fluorescence signal can still be overwhelmed by background signal variation. This is not the case for short, 1ms gate widths utilizing room light based triggering where standard deviation of background signal is less than 7% of fluorescence signal at 0.25 nM.
Fig. 6. In (a) the normalized standard deviation values of the background signals in the 700 nm channel are shown for a variety of image acquisition settings (from 30 to 50 images each). In (b) the same standard deviations are shown, but normalized by the fluorescence signal. It can be seen that while standard deviation as a fraction of background signal as seen in (a) may be quite low for some of the longer exposure times, this is not the case when considering standard deviation as a fraction of fluorescent signal which is the more relevant metric. This is a result of the much lower fluorescence to background ratio seen at longer imaging times.

While images acquired at 40 ms gate widths and lower show standard deviations that are more than 30% of mean fluorescence signal, those at 100 ms are considerably lower and as such 100 ms or longer gate widths might be considered for FGS under these conditions. The results of using extended acquisition times can be seen in Fig. 7 where error bar plots for 30-50 images taken at 40 ms (Fig. 7(a)) and 100 ms (Fig. 7(b)) gate widths are compared to those taken using room light based triggering and in the dark (both at 1ms) (Fig. 7(c)). While the 40 ms gate width images show inferior detection capabilities as was expected those at 100 ms are comparable to the 1 ms gate width images that utilize room light based triggering. Percent standard deviations for background subtracted signals at 100 ms gate widths remain below 3% which is very close to the 2% maximum seen when room light based triggering is used.

Fig. 7. Detection in OR under different imaging parameters at the 700 nm channel. (A,B&C) Error bar plots showing mean and one standard deviation above and below the mean for background subtracted signal for series of 30-50 images taken at each IRDye 680RD concentration from 0 to 0.98 nM. (a) Images taken in the surgical OR with no room light based triggering, 40 ms gate width and no camera gain. (b) Images taken in the surgical OR with no room light based triggering, 100 ms gate width and no camera gain. (c) Images taken in the surgical OR with room light based triggering (offset left / blue) and images taken in complete darkness (offset right / black). Both sets of images taken using 1ms gate widths, full camera gain and 10x overdriving of the LEDs.
3.3 Tissue phantom testing: gated acquisition with and without room light based triggering at 800 nm

Fluorescence detection levels in the surgical OR (surgical room 1) at the 800 nm channel were tested in the same manner as those described previously for the 700 nm channel. Serial dilutions of IRDye 800CW (LICOR Biosciences, Lincoln, NE) (1% Intralipid and 0.01% India ink, for $\mu_a = 0.02 \text{ mm}^{-1}$ and $\mu_s' = 1.0 \text{ mm}^{-1}$) were examined under a variety of acquisition settings.

Despite reduced background light signal at the 800 nm channel as compared to the 700 nm channel, the same problem created by the large 120Hz fluctuations makes standard sub-millisecond pulsed imaging impractical at lower fluorophore concentrations. This can be seen in Fig. 8(a), where signal is seen to have a high variance just as was seen at the 700 nm channel in Fig. 4(a). Room light based triggering was again able to alleviate this problem as illustrated in Fig. 8(b), where the same acquisition settings were used but this time triggering was either based on room light signal (offset left/blue) or imaging was done in complete darkness (offset right/black). The situation is similar to what was seen at the 700 nm channel where again, reduction in signal variation is drastic and the background subtracted signals using room light based triggering are comparable to those seen when imaging in a completely darkened room.

![Fig. 8. Detection in OR with and without room light based triggering at the 800 nm channel. (A&B) Error bar plots showing mean and one standard deviation above and below the mean for background subtracted signal for series of 50 images taken at each IRDye 800CW concentration from 0 to 3.9 nM. This is for 1ms gate widths, full camera gain and 10x overdriving of the LEDs. (a) Images taken in the surgical OR with no room light based triggering. (b) Images taken in the surgical OR with room light based triggering (offset left / blue) and images taken in complete darkness (offset right / black).](image)

In order to provide a more visual illustration of these results, actual background subtracted phantom images are presented in Fig. 9. Phantom images over the range of concentrations are shown. These are single representative images taken from the series of images used to provide the data displayed in Fig. 8.
Fig. 9. Single fluorescence background subtracted images of approximately 1.6cm by 1.6cm phantoms taken with 1ms gate widths and full camera gain. Images were taken separately and have been stitched together for viewing purposes. (a) Images taken without room light based triggering clearly demonstrate the inability to visualize fluorescence with strong variations in signal due to room light fluctuation. In (b) images taken with room light based triggering demonstrate significant fluorescence visualization at the higher concentrations examined. In (c) images taken in the absence of room lights show comparable fluorescence visualization to those seen in (b).

While background light fluctuations at the 800 nm channel are still significant enough to interfere with sub-millisecond pulsed light imaging in the absence of room light based triggering, overall signal in this channel is reduced by more than an order of magnitude as compared to the 700 nm channel. Background and fluorescence signals for the 1ms acquisition settings as explained previously, both with and without room light based triggering as well in the dark are shown in Fig. 10(a) for a 0.25 nM concentration of IRDye 800CW. As a result of these lower background signals, the use of longer acquisition times at the 800 nm channel may show greater promise than at lower wavelengths. Acquisition times were increased to 10 and 20 ms while camera gain was maintained at its maximum and the resulting signal contributions for the same 0.25 nM phantom are seen in Fig. 10(b). The problem is that background variation is still quite large in relation to fluorescence signal contribution even at these longer 10 and 20 ms gate widths. In Fig. 11(a) the standard deviations from 30 to 50 background images using various acquisition settings are divided by the corresponding average background signal and compared. While in Fig. 11(b) the standard deviation from background images divided by the fluorescence portion of signal for the 0.25 nM IRDye 800CW phantom. The results are similar to what was seen in the 700 nm channel where there is a clear advantage to pulsed imaging at 1 ms using room light based triggering.

Fig. 10. (a) Average background and fluorescence signal levels in the 800 nm channel are shown (from 50 images) of 0.25 nM IRDye 800CW phantom all acquired using 1ms gate width, full camera gain, and 10x LED overdriving. Center and left bars are from images acquired in full surgical OR background light with and without room light based triggering respectively. Right bar is for images acquired in complete darkness. (b) Average background and fluorescence signal levels from 50 images of 0.25 nM IRDye 800CW phantom at various gate widths all using full camera gain, but no LED overdriving. All error bars represent a single standard deviation.
Fig. 11. (a) The normalized standard deviations of background signals at the 800 nm channel are shown, for a variety of image acquisition settings (30-50 images at each). Acquisition settings are shown below each bar. (b) The same standard deviations are shown, but normalized by the fluorescence contribution calculated from a 0.25 nM IRDye 800CW phantom. It can be seen that while standard deviation as a fraction of background signal as seen in (a) may be quite low for some of the longer exposure times this is not the case when considering standard deviation as a fraction of fluorescent signal which is the more relevant metric. This is a result of the much lower fluorescence to background ratio seen at longer imaging times.

4. Discussions

Fluorescence imaging for surgical guidance within a brightly lit OR presents a number of challenges that are not present in other imaging scenarios. The relative fraction of procedures done in an open environment is not likely to increase given the advances in minimally invasive surgery, however, the number of fluorescence guided surgeries is increasing and open procedures are a key part of oncologic surgery. Several factors contribute to the detection capabilities and quality of background subtracted images that can obtained in a brightly lit open OR procedure. The ability to achieve video rate imaging is extremely important as this is the current norm for FGS and surgeons are unlikely to accept anything less. However, as high background signal in relation to fluorescence signal necessitates the use of background subtracted images this becomes more difficult. In this scenario video rate requires 60 frames per second (fps) rather than the usual 30 fps. Even so this may not be the driving force for limiting gate widths and acquisition times. Successful background subtraction requires that the only difference between background and fluorescence images stem from the excitation light present during the fluorescence image acquisition. In a well-lit OR where background light signal, even when using room light based triggering, will generally be greater than fluorescence signal, variations across the imaging field which occur due to both variations in tissue optical properties and field inhomogeneity must be corrected for. Background subtraction provides a relatively simple method of doing this. Considering a surgical environment in which movement from the surgeon or others around the FOV has the potential to create shadows, in addition to the fact that the surgeon will actually be manipulating tissue, it is critical that image sets be acquired rapidly if background subtraction is to be successful. As such even though the 100 ms acquisition times as seen in Fig. 7(b) may enable detection in the static testing environment used in this study, they are not practical in an actual surgical environment. Images taken at these longer exposure times would likely suffer degraded performance due to changes in the imaging field during acquisition. An alternative to the use of longer imaging times that encompass multiple periods could be to select acquisition times that are multiples of the room light period as this would also be expected to reduce signal variation.
Increasing fluorescence signal in relation to background signal is important for minimizing the influence of inevitable variations in background signal on the final background subtracted images that are displayed. A change in acquisition time will have the same effect on both background and fluorescence signal and so in and of itself does not provide a method of doing this. An alternative proposed method for signal removal of background ambient light is to modulate the beam at high frequency and sample the signal with this same demodulation. This process pioneered by Zhu et al. [21, 22] allows fast capture although it can suffer from dynamic range limitations if the room lights are a major contributor to the overall detected intensity. The use of short (sub-millisecond) gate widths and room light based triggering allows images to be acquired during background light minimums which is beneficial in the context of dynamic range limitations. This enables increased fluorescence to background ratios and also has the potential to further improve system performance by enabling longer imaging times, increased camera gain, or greater excitation power before saturation occurs. Additionally, the use of short gate widths enables overdriving of LEDs which further increase fluorescence to background ratios.

The reduction in background light variation is essential for imaging in the brightly lit OR. While longer acquisition times provide a means of doing this they are impractical for FGS. The alternative of minimizing background signal fluctuation using room light based triggering enables drastic reductions in signal fluctuations. The use of short, sub millisecond gate widths and room light based triggering present a number of other advantages for FGS and really may well represent the most promising approach. It should also be noted that it may be possible to achieve the same results via triggering directly off of the line voltage driving the room lights rather than the room lights themselves.

Background subtraction is essential for enabling room light based triggering to compare with imaging in the absence of ambient light. While the signals displayed for the two techniques shown in Fig. 3 are comparable, it must be realized that this is following background subtraction. In reality, the signals recorded under ambient light for both the fluorescent image and the background image are considerably higher than those seen in the darkened room. It is only after the background image is subtracted from the fluorescence image that the two become comparable. In a dark room or under lower intensity ambient light, background subtraction is less critical or even unnecessary as the background signal is generally so much lower than the fluorescence signal that variations are inconsequential. That is not the case in the well-lit OR where background light signal, even when using room light based triggering, will generally be greater than fluorescence signal and so variations across the imaging field which occur due to both variations in tissue optical properties and field inhomogeneity must be corrected for. Background subtraction provides a relatively simple method of doing this. However, it also must be kept in mind that image gate widths as well as the time between background and fluorescence acquisitions can have a significant influence on the quality of background subtracted images.

This study has demonstrated that pulsed light imaging gated to the background light signal can reduce background light signals that cause variation in the image, and improve the potential for pathologically relevant fluorophore concentrations to be detected in room light conditions and with a detection level that is comparable dark room conditions. At this time, it is apparent that several commercial systems have now developed some kind of gated acquisition such as shown here, however few have developed it to be sensitive to the room light environment. The improvement in sensitivity as a result of room light based gating can be imperative in a range of specific conditions. Most of the logistics as to how this could be implemented have been worked out here and the demonstration using tissue phantoms has shown the gain possible in dynamic range from this method.

Methods to remove background signal also stand to aid in the exploitation of natural tissue auto fluorescence. The use of auto fluorescence to differentiate tissue types continues to be an active area of research and is especially relevant considering the serious concerns regarding toxicity as well as the lengthy and costly approval processes for any exogenously administered agent. The techniques used herein would certainly be applicable to these
approaches despite that fact that auto fluorescence signals are typically significantly lower. The results presented here demonstrate that pulsing to the room light signal can reduce background light signal variation to the levels wherein other factors such as excitation light leakage tend to become the limiting factor.

5. Conclusions
The performance of the pulsed light system was evaluated under a variety of background light conditions. The system is capable of imaging under the less intense background light conditions found in standard laboratory setting. However, background fluorescent light levels found in an actual OR are substantially higher and demonstrate large amplitude changes at a frequency of 120Hz. Under these conditions sub-millisecond fluorescent imaging is hampered by extreme fluctuations in detected background signal as compared to fluorescence signal. While longer exposure times which allow the periodic fluctuations in background signal to be averaged out can enable fluorescence imaging under these conditions they are not practical for FGS. The technique of using the periodic room light signal to trigger image acquisition has been shown to drastically reduce background signal fluctuations as well as enable images to be acquired at the background light minimums. The technique performs so well that background subtracted images acquired using 1ms gate widths, full camera gain, and maximum LED overdriving are comparable to those acquired in a completely dark room. The use of room light signal to trigger image acquisition will enable FGS to be performed using pulsed light in a brightly-lit operating room with minimal impact on performance.

Funding
National Institutes of Health (R01CA109558 and R01CA167413).