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Determining the Performance of Fluorescence Molecular Imaging Devices using Traceable Working Standards with SI Units of Radiance

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Abstract

To date, no emerging preclinical or clinical near-infrared fluorescence (NIRF) imaging devices for non-invasive and/or surgical guidance have their performances validated on working standards with SI units of radiance that enable comparison or quantitative quality assurance. In this work, we developed and deployed a methodology to calibrate a stable, solid phantom for emission radiance with units of $\text{mW} \cdot \text{sr}^{-1} \cdot \text{cm}^{-2}$ for use in characterizing the measurement sensitivity of ICCD and IsCMOS detection, signal-to-noise ratio, and contrast. In addition, at calibrated radiances, we assess transverse and lateral resolution of ICCD and IsCMOS camera systems. The methodology allowed determination of superior SNR of the ICCD over the IsCMOS camera system and superior resolution of the IsCMOS over the ICCD camera system. Contrast depended upon the camera settings (binning and integration time) and gain of intensifier. Finally, because of architecture of CMOS and CCD camera systems resulting in vastly different performance, we comment on the utility of these systems for small animal imaging as well as clinical applications for non-invasive and surgical guidance.

Index Terms

Molecular and cellular imaging; Optical imaging/OCT/DOT; Evaluation and performance; System design; Validation

I. Introduction

Near-infrared fluorescence (NIRF) imaging emerged from the biomedical optics field approximately twenty years ago, and today there are several commercial systems both for small animal imaging [1, 2], as well as clinical imaging [3–8]. The technique consists of administering a near-infrared fluorophore which, when excited, produces a fluorescent signal that is multiply scattered through tissues and when re-emitted, collected using an imaging system. By using tissue penetrating, near-infrared light of 750 nm or greater wavelength for excitation, endogenous autofluorescence can be avoided and signal from the fluorophore, typically of 780 nm or greater wavelength, can be imaged from as deep as 3–4 cm [9]. Despite significant progress in translating NIRF imaging from preclinical to clinical applications, there remains no working standards with the International Systems of Units (SI units) of emission radiance to quantify device sensitivity and enable non-clinical testing of device performance at known levels of radiance. Today, most devices employed for translational and clinical NIRF imaging depend upon the ability to detect indocyanine green (ICG) in humans. Yet substantial differences between device designs result in a wide variation of performance [7]. Quantifying device performance at relevant radiance levels is critical for translational NIRF molecular imaging using molecularly targeting moieties conjugated to NIRF dyes. Without a working standard to delineate device performance through non-clinical testing, there is no means to determine whether *in vivo* NIRF imaging of a contrast agent is limited by device performance or by the specificity of the contrast agent for its disease target.

Using a series of stable, solid phantoms, Zhu and coworkers [10] compared the performance of various detectors to show dramatic differences in contrast and signal-to-noise ratio (SNR), but the solid phantoms were not calibrated in SI units of output radiance so that measurements could not be recapitulated by others using different phantoms and calibration schemes. Likewise, in pioneering work to translate a first-in-humans, NIRF-labeled EGFR targeting monoclonal antibody, Rosenthal and colleagues labeled Cetuximab with an NIRF dye, IRDye800, and used a combination of commercial NIRF imaging systems, including the SPY (Novadaq Technologies Inc., Toronto, ON, Canada) and Pear Impulse Small Animal Imager (LI-COR Biosciences, Lincoln, NE), to show that the detection of tumor margins also differed between devices [11]. Because there are no working standards of emission rates in reportable SI units of radiance, there are also no non-clinical tests to compare devices from the same manufacturer, let alone perform quality assurance in a traceable fashion as required for marketing by the Food and Drug Administration (FDA). This is especially important for surgical guidance, because the ability of a device to detect the signal from a NIRF imaging agent can critically impact the ability of the surgeon to accurately identify and remove cancerous tissues without removing excessive amounts of normal tissues.

In this work, we devise methodologies for non-clinical testing of two NIRF molecular imaging devices using working standards that have known and stable emission rates reportable in SI units of radiance. Previously [10], we found that the QDots 800 embedded in a solid polyurethane and titanium dioxide media can provide a working standard that, unlike commercial phantoms that employ organic dyes such as Alexa Fluor 750 (Caliper,

Hopkinton, MA), is stable over time. Herein we demonstrate the use of established radiometry principles to assign SI units of radiance to the standard tissue phantom, and then use this as a working standard to assess device performance metrics of signal-to-noise, contrast, and resolution at excitation irradiances we have found in investigational human studies. Using these working standards we then compare the performance of an investigational imaging device deployed in clinical lymphatic studies [7] with a new camera system based upon a military grade intensifier fiber coupled to a scientific CMOS. Given that the FDA guidelines require quantification of performance using SI or SI derived units, we believe that the adoption of traceable working standards will be essential to advance the technological developments of NIRF molecular imaging devices and imaging agents for both clinical and preclinical applications. Other clinical fluorescence imaging systems such as autofluorescence bronchoscopy, autofluorescence endoscopy, chromoendoscopy, fluorescence angiography, oral cancer screening, skin cancer screening, and others could also benefit from the measurements made on the working standards with the SI units of radiance.

II. Materials and methods

A. Calibration of QDots 800 based luminescent solid tissue phantoms for use as working standards

The tissue phantoms consisted of a strip of 33 mm² wells from a 96 well plate filled with incremental amounts of QDots 800 nanoparticles added to a polyurethane solvent containing TiO₂ scattering particles to exaggerate the scattering properties of tissues [10]. The final concentration of QDots 800 in the wells ranged from 0 to 10 nM. The preparation of the tissue phantoms is described elsewhere and has been shown to have stable photon emission rates [10]. Although QDots 800 do not have a substantial absorbance or emission spectra in the near-infrared wavelengths, there is a sufficient absorption cross section at 785 nm and luminescent yield at 830 nm to mimic trace amounts of ICG or IRDye800 as we previously described. In this work, our first goal is to assign SI units or SI derived units of radiance to each well of the QDots 800 tissue phantom using radiometry techniques, so that they become traceable working standard with which to evaluate device performance.

In order to ascribe radiance ($\text{mW} \cdot \text{sr}^{-1} \cdot \text{cm}^{-2}$) in SI units to the luminescence emitted from the surface of each well of the tissue phantom, its output has to be compared to a reference source with known radiance. The reference source is a one-inch diameter integrating sphere (RAA4, Internal Light Tech, Peabody, MA) into which variable levels of 830 nm diode laser (HL8338MG, 830 nm, 50 mW, Thorlabs, Newton, NJ) light is input via optical fiber, controlled by the diode laser drive current. The integrating sphere output port measures 34 mm², closely matching the area of the tissue phantoms. We used the variable focus ICCD system (described below in Section IIC) as a transfer detector, detecting signals from the integrating sphere source and the tissue phantoms alternately. The camera focus lens of the ICCD system was positioned at a fixed 37 cm working distance from the output port of the integrating sphere (Fig. 1(a)), and images of the integrating sphere output port at the focal plane were acquired on the ICCD as a function of laser diode driving current. We increment the driving current of the laser diode to generate average ICCD intensity signal values with

arbitrary unit (AU) from the pixels corresponding to the source being imaged that span the signal ranges detected from the tissue phantoms with varying QDots concentration. At each drive current step, the ICCD intensity signal from the integrating sphere source is recorded and its radiance ($\text{mW} \cdot \text{sr}^{-1} \cdot \text{cm}^{-2}$) is calibrated. Figure 2 illustrates the configuration in this particular calibration, according to radiometric principles. Radiance from the integrating sphere source S_R with exit port planar area of 34 mm^2 is determined from the irradiance E measured by a National Institute of Standards and Technology (NIST) -calibrated Si photodiode power meter D_R (S2281, Hamamatsu, Bridgewater, NJ), which has a 50 mm^2 photoactive area at a distance d of 4.46 mm from the S_R exit port. The solid angle subtended by D_R on S_R (describing how much of the detector sees of the source) is calculated using the spherical cap area of A_{S_R} at distance d rather than the planar area of the exit port due to the short measurement distance. The measured irradiance E can then be converted to radiance by dividing it by the solid angle, ω_1 .

Figure 1(b) illustrates the illumination and imaging of the tissue phantoms positioned in the focal plane again located 37 cm away from the camera lens and illuminated with uniform 785 nm laser diode (HPD1005-9mm-78503 model, High Power Devices Inc., NJ). The uniform illumination was implemented by placing a diffuser in front of the laser output and uniformity was confirmed by measuring incident excitation irradiance. Since the now-calibrated sphere source, through control of the laser diode drive current, generates the same ICCD intensity signal levels as the tissue phantom at a specific 785 nm excitation irradiance ($\text{mW} \cdot \text{cm}^{-2}$), and they are of similar size, both output at 830 nm and measured at nearly the same time using the same detection configuration and camera settings (which included the gain of the intensifier and CCD, integration time, camera elements, and binning of the CCD output), the radiance ($\text{mW} \cdot \text{sr}^{-1} \cdot \text{cm}^{-2}$) calibration of the reference sphere source is therefore ascribed to the tissue phantom. Additionally, since fluorescence/luminescence radiance increases linearly with incident 785 nm excitation irradiance ($\text{mW} \cdot \text{cm}^{-2}$) in a Beer-Lambert fashion for dilute emitters, the radiance from the tissue phantoms as the excitation irradiance is varied from 1.0 to $7.0 \text{ mW} \cdot \text{cm}^{-2}$ is determined using the same procedure using the reference source and transfer detector. Hence, the tissue phantoms acquire radiance units normalized to the irradiance of the excitation illumination. In practice, since the tissue phantoms have been calibrated for radiance at emission per irradiance at excitation, the radiance of the tissue phantom can be known after measurement of the excitation irradiance on the object focal surface at the time of collection. The calibrated tissue phantoms can now be used as working standards, to evaluate performance of NIRF imagers either as a total system or the optics or sensor separately, using known rules of radiometry[12, 13].

B. Figures of merit for fluorescence molecular imaging

Signal-to-noise ratio and contrast—To quantify the measurement sensitivity of NIRF imaging systems, the QDots 800 based solid working standard was placed at the imager's object focal plane, as shown in Fig. 1(c). The SNR corresponding to each well in the working standard was calculated by employing the average intensity and standard deviation (σ) of values across pixels corresponding to each well area as it is done in nuclear imaging [14]:

$$SNR = \frac{\bar{S}^t(C) - \bar{S}^b(0)}{\sigma} \quad (1)$$

where $\bar{S}^t(C)$ and $\bar{S}^b(0)$ are the AU values for the pixels corresponding to the entire well containing C concentration of QDots 800 and to the well without QDots 800 respectively, and σ is the standard deviation of AU values in the well without QDots 800. SNR was then plotted as a function of radiance ($\text{mW} \cdot \text{sr}^{-1} \cdot \text{cm}^{-2}$) of each well in the working standard. The contrast was computed by:

$$\text{Contrast} = \frac{\bar{S}^t(C)}{\bar{S}^b(0)} - 1 \quad (2)$$

and plotted as a function of radiance ($\text{mW} \cdot \text{sr}^{-1} \cdot \text{cm}^{-2}$) of each well in the working standard.

Resolution—To assess the spatial resolution of NIRF imaging system, a negative USAF 1951 test target (USAF-1951, NT53-714, Edmund Optics) was placed at the output aperture of a 6 inch diameter integrating sphere (Oriel, 70451) illuminated with an 830 nm laser diode (HL8338MG, 830 nm, 50 mW, Thorlabs, Newton, NJ) as shown in Fig. 3. The 830 nm diode driving current was adjusted to radiances corresponding to those measured from the QDots 800 based solid working standards. The images of the negative USAF 1951 test target were acquired at the object focal plane. The contrast transfer function (CTF) was calculated from the cross-sectional intensity profiles of three horizontal and vertical lines in each numbered bar group with the following equation:

$$CTF(f) = \frac{S_{\max}(f) - S_{\min}(f)}{S_{\max}(f) + S_{\min}(f)} \quad (3)$$

where $S_{\max}(f)$ and $S_{\min}(f)$ are the upper and lower bounds of the intensity signal (in AU) of adjacent line patterns at a corresponding spatial frequency of each bar element f (lines/mm). The transverse or lateral resolving power can be calculated by considering the Rayleigh criterion, which defines the resolution as the distance between two points that are resolved when the first minimum of one Airy disk is aligned with the central maximum of the second Airy disk. Under this limit, the Rayleigh criterion separation distance typically corresponds to a value of 26.4% [15].

C. NIRF imaging systems

In our investigational NIRF imaging system, we employed a laser diode (HPD1005-9mm-78503 model, High Power Devices Inc., NJ) operating at 785 nm wavelength, whose output first was collimated, and then outfitted with a 785 nm band pass “clean-up” filter (LD01-785/10, optical density > 5 at 705–765 nm and 803–885 nm, Semrock, Inc.) and finally expanded by an optical diffuser. The emission light was collected

after it passed through two 830 nm band pass filters (830FS10, optical density >5 at 785 nm, Andover, Salem, NH) separated with the Nikon focus lens (AF NIKKOR 28 mm f/2.8D, Nikon, NY, USA) to increase the optical density at the excitation wavelength in order to reduce the leakage of excitation light. The filtered fluorescence signals were focused onto a Gen III image intensifier coupled with a CCD camera (a customized fiber optic coupled CCD camera with e2v CCD47-20 back-illuminated chip, Princeton Instruments, Trenton, NJ). The pixel size of the CCD is $13 \mu\text{m} \times 13 \mu\text{m}$. To assess the performance of IsCMOS camera system, we replaced the CCD camera with a scientific CMOS camera (Zyla 5.5 sCMOS, ANDOR, Concord, MA), leaving all other components and radiometric arrangements unchanged. The pixel size of the IsCMOS is $6.5 \mu\text{m} \times 6.5 \mu\text{m}$. To acquire images for comparison, the ICCD was binned by 2 and the IsCMOS was binned by 4 to obtain an effective pixel size of $26 \mu\text{m} \times 26 \mu\text{m}$. The ICCD and IsCMOS cameras were cooled down to -25°C and 0°C , respectively, as suggested by the manufacturers and the FOV was adjusted to 10 cm in diameter. The control of devices and data acquisition were implemented under a LabVIEW based interface (National Instruments, Austin, TX).

III. RESULTS

A. Radiance of QDots 800 based solid phantom in SI units

Figure 4(a) shows the radiometric-specific function curve that relates the ICCD output of AU values, to the calibrated 830 nm radiance ($\text{mW} \cdot \text{sr}^{-1} \cdot \text{cm}^{-2}$) from the output aperture of the integrating sphere. Figure 4(b) illustrates the ratio of emission radiance at 830 nm to the incident 785 nm excitation irradiance for each of the wells containing different concentrations of QDots 800. The plot shows that the ratio of emission radiance to excitation irradiance is proportional to the QDots 800 concentration and that the linear relationship is preserved as the excitation irradiance increases from 1.0 to $7.0 \text{ mW} \cdot \text{cm}^{-2}$, which represents the incident excitation irradiance used by most investigational and clinical NIRF imaging devices [7]. At the lower and upper ranges of 830 nm radiance, the radiometric-specific function curves are shown in Fig. 5(a) and (b), respectively, demonstrating the dark noise of the detector and maximum measurable radiance prior to camera saturation.

B. Measurement sensitivity of ICCD and ICMOS based NIRF imaging systems

Figure 6(a) shows the images of same working standard acquired by the IsCMOS camera system as compared to that acquired by the ICCD camera system at various integration times, intensifier gains, and binning modes but at the same uniform excitation irradiances of $1.9 \text{ mW} \cdot \text{cm}^{-2}$. These images are reported in terms of AU to highlight that different radiometric calibrations or *function curves* are needed for the different cameras at differing camera settings as shown in Fig. 6(b). Figure 6(b) provides the radiometric-specific relationship to calibrate camera output of AU units to $\text{mW} \cdot \text{sr}^{-1} \cdot \text{cm}^{-2}$ radiances at the object focal plane. Because of the linear relationship between $\bar{S}(C)$ reported in AU and radiance reported in $\text{mW} \cdot \text{sr}^{-1} \cdot \text{cm}^{-2}$ (shown in Fig. 4(a) and 6(b)), $\bar{S}(C)$ can be represented in AUs or in SI units of $\text{mW} \cdot \text{sr}^{-1} \cdot \text{cm}^{-2}$. At 200 ms full frame acquisition, Figures 6(a) and (b) show the IsCMOS camera recorded higher AU values and is more sensitive on the basis of the slope of the AU versus 830 nm radiance function curve when compared to the ICCD camera. At integrating times of 33 ms and intensifier gain of 800 V,

the emission AU values of the acquired images by IsCMOS and ICCD camera systems and their associated sensitivities become comparable. The plots of the SNR and contrast as a function of 830 nm radiance at the full frame mode, the respective binning modes, and various integrating times are shown in Fig. 7. Upon using the full frame, increased integration time to 200 ms, and lower gain of the intensifier, the ICCD camera system has higher SNR (Fig. 7(a)), but the IsCMOS camera system gives better contrast (Fig 7(c)). Upon binning, and using reduced integration time and increased intensifier gain, the ICCD camera system is superior in both the SNR (Fig. 7(b)) and contrast (Fig 7(d)).

C. Resolution of ICCD and IsCMOS based NIRF imaging systems

Figure 8 shows images of the negative USAF 1951 standard resolution target acquired using the ICCD and IsCMOS camera systems at the full frame and binning modes at 830 nm radiance of $1.94 \times 10^{-6} \text{ mW} \cdot \text{sr}^{-1} \cdot \text{cm}^{-2}$. From Fig. 8 (a)–(d), one can conclude that the images acquired using the ICCD camera system have a lower spatial resolution than those acquired using the IsCMOS camera system, and that binning results in reduced resolution as expected for both cameras. As discussed below, the differences in chip read-out architecture (frame transfer read-out versus single pixel readout) during a shutterless, photoactive acquisition can lead to smearing and reduction in resolution. Figure 9 depicts the CTF calculated from the ICCD image of Fig. 8(a) as a function of the spatial frequency f along the horizontal and vertical directions, respectively. From the horizontal and vertical CTF curves, we found that a contrast value of 26.4% corresponds to the line pairs of 1.55 and 1.38 lp/mm, respectively, indicating that the transverse and lateral resolutions of the ICCD system are 323 μm and 362 μm , respectively, from the half-width between two line pairs. Using other images shown in Fig. 8(b)–(d), the corresponding transverse and lateral resolutions were also determined. Table 1 shows that the IsCMOS camera system has higher resolution compared with that of the ICCD camera system at the full frame and binning modes. In addition, there is no significant impact on the resolutions by varying 830 nm illumination radiance within the range corresponding to those measured from the working standard (data not shown for brevity).

IV. DISCUSSION

IEC 60601 is an international series of technical standards that requires numerical indications of performance of medical devices be in SI units or SI-derived units for traceability. The pathway to metrological traceability to the SI units is not always obvious especially in applications such as NIRF molecular imaging where both the molecular imaging agent and the imaging device are each subject of continuing technological improvements, in addition, most imaging systems have many parameters that can be varied (such as $f/\#$, focal length, detector gain, etc.) and that varying imaging parameters will directly affect system response, such as was measured in this study; but it is in such complex applications that it becomes essential to establish methods for evaluating performance of the device as a light measuring instrument, in order to guide improvements efficiently and in the process, assess imaging agent performance objectively. Traceability of measurements to the SI assures that the radiometric measurements, when performed properly, can be compared across device systems, across locales, and across time. In this application, we calibrated

stable, luminescent working standards with the effective shelf life of more than 2 years to SI units of radiance by first using a NIST calibrated power meter and an integrating sphere source to calibrate the AU output of an ICCD camera system configured under specific radiometric conditions, and then secondly, using the AU output of that same system under same conditions to assign a radiance ($\text{mW} \cdot \text{sr}^{-1} \cdot \text{cm}^{-2}$) scale to the tissue phantoms working standards. The measured ratios of emission radiances ($\text{mW} \cdot \text{sr}^{-1} \cdot \text{cm}^{-2}$) at 830 nm per 785 nm excitation illumination irradiance ($\text{mW} \cdot \text{cm}^{-2}$) from standards with 2.0 nM-10.0 nM concentrations of QDots 800 ranged from 5.2×10^{-7} to 2.6×10^{-6} 830 ($\text{mW} \cdot \text{sr}^{-1} \cdot \text{cm}^{-2}$)/785($\text{mW} \cdot \text{cm}^{-2}$). We then used these working standards to evaluate overall device performance as we changed the detector from a CCD to a sCMOS chip and operated the intensified systems under different operating conditions. Radiance is a property of the light source and is invariance with distance. This allows the radiance calibration value to be used in other optical configurations.

It is especially noteworthy that we do not attempt to convert the AU output of ICCD or IsCMOS-based devices to the SI units. The AU output is an aggregate value of the sensor's conversion of detected photoelectrons and a function of all components of the imaging device and their relationship to the fluorescent/luminescent object being imaged. When camera and/or intensifier gain increases, integration time increases, or working distance between camera and object decreases, the AU output signal changes, even though the radiance of the working standard remains fixed. Instead we generated a working standard of known and constant radiances reported in SI units so that we can report the performance of the *entire* device in terms of sensitivity (Fig. 6(b)), SNR (Fig. 7(a) and (b)), contrast (Fig. 7(c) and (d)), and resolution (Table 1) at specified and known radiance levels *at the object focal plane*. In this manner, we can use the working standard across most devices of varying design to assess performance in a traceable manner. While some commercial imaging devices of fixed optical paths report camera output in terms of $\text{photons} \cdot \text{sec}^{-1} \cdot \text{cm}^{-2} \cdot \text{sr}^{-1}$, these quantities reflect what is measured at the detector and are not the radiance of the desired object in the scene being imaged. Our methodology provides a working standard of known radiance for non-clinical testing of overall device performance that answers the question *can the device be operated so that it can detect fluorescent radiance of a known quantity?* Although we demonstrated the use of the working standard to evaluate performance upon change of detector from an ICCD to IsCMOS in an otherwise identical optical system, the working standard could also be used to assess changes in overall device performance with altered components (lens, optics, laser diodes) and with change in parameters of operation as shown at different binning mode, gain setting and integration times in Fig. 7.

The use of the working standards showed that that ICCD camera system has overall superior in SNR, but is inferior in resolution when compared to the IsCMOS camera system, even when the two systems are binned for similar pixel size. The difference in resolution can be attributed to the different architectures of CCD and CMOS chips. For a typical frame transfer CCD, each pixel is composed of a photodiode along with an adjacent charge transfer region arranged in a column to form a vertical charge transfer register. The collected charges in each pixel are transferred to the adjacent charge transfer cells in the columns simultaneously. One row of data is transferred to a separate horizontal charge transfer

register, which is then read out serially and sensed by a charge-to-voltage conversion and output amplifier. This process is repeated until all rows are read out. This architecture produces a low-noise, high-performance imager, and if during readout, the CCD remains photoactive, then smearing artifacts occur as shown in Fig. 8(a) and (c), which adversely affects the resolution. In comparison, each CMOS's pixel contains a photodiode, charge-to-voltage conversion and amplifier sections. The column output signal is connected to a set of decode and readout electronics, which are arranged for each column outside the pixel array. This architecture allows the pixel signals from the entire array to be read out independently and at the same time. Hence, there are no smearing artifacts, as shown in Fig. 8(b) and (d), for a photoactive array to impact the resolution. The lower SNR for the IsCMOS system as compared to the ICCD system may be due to the higher pixel to pixel variation in background noises in the sCMOS chip as a result of dark current, hot blemishes, and other spurious noises that can vary widely across the sCMOS chip. While cooling and onboard spurious noise filtering greatly reduce the impact of these noise sources on the images, they still result in a higher standard deviation in the background signal which results in a decrease in the SNR as calculated using Eq. (1). Based upon our previous study [10], the SNR of 5 represents the detection limit of NIRF intravital imaging systems. Although the SNR of IsCMOS system is less than that of ICCD system, it is greater than 5. The impact of this SNR reduction is likely to be negligible in clinical practice. In addition, the architecture of CMOS chirp results in a short readout time, allowing for ultrafast imaging that is necessary for sensitive, intraoperative and non-invasive imaging.

Our focus herein has been to define performance of NIRF imaging systems for NIRF labeled targeted, fluorescent cancer imaging agents. Despite the intense research and development of "first-in-humans," agents and the known safety and toxicity profiles for the NIRF fluorophore IRDye800 [16], only one NIRF imaging agent has been translated into the clinic [11]. Thus far, there has been a number of NIRF imaging devices translated in the US, Europe, and Asia, to image non-targeting ICG in humans, but their designs vary widely with no quantification of performance. The doses of ICG detected with each device also varies, suggesting that variable design can impact sensitivity [7]. Since molecular imaging with NIRF depends upon adequate sensitivity to detect concentrations relevant to overexpressed disease markers, variable device sensitivity could translate into the inability to detect emerging "first-in-humans" agents at low concentrations, requiring increased dosing levels and, depending upon pharmacokinetics and clearance, reduced performance. For example, antibodies, which have high binding affinities that are advantageous for molecular imaging but have long circulation times that result in high background tissue levels have been employed in nuclear, NIRF, and dual-labeled nuclear/NIRF preclinical imaging studies [17]. While the sensitivity of nuclear imaging enables trace dosing, the long circulation time of antibodies coupled with the radioisotope half-life consideration can limit nuclear imaging with radiolabeled antibodies. With the lack of a half-life associated with nuclear imaging studies, NIRF labeled imaging agents are well suited for surgical guidance if the device can detect small quantities of agent. However, if higher than trace doses are required by the imaging device to detect NIRF labeled antibodies, then the background signals from their long circulation times reduces contrast and can limit performance. Despite the universal importance of defining the sensitivity of molecular imaging devices, unlike the nuclear

imaging field, there has been no standards to assess the performance of fluorescence imaging devices. As a result, in the US, all fluorescence molecular imaging agents must be approved specific to a manufacturer's device as a combinational product. The regulatory pathway to approve a fluorescent imaging agent specific for a device is necessitated by the lack of standards in the area of fluorescence biomedical imaging and impedes the clinical adoption of technologies that ultimately improve patient health and reduce health care cost.

Finally, we identify drawbacks to our methodology for assessing overall device performance with SI-traceable working standards. First of all, QDots are luminescent but may not always be used as working standards for conventional frequency- or time- domain fluorescence imaging measurements because their radiative decay constants are greater than tens of nanoseconds as compared to sub-nanosecond fluorescence lifetimes. Nonetheless, in continuous wave measurements, when QDots are used as a luminophore they can provide a stable emission rate that can be used to assess overall fluorescent device performance. Secondly, the excitation and emission spectra of QDots are broad and, in the absence of incident excitation light but under ambient light conditions, will luminesce and may lead to falsely low performance metrics if not deployed as a working standard within a dark-room setting. Because device performance should be assessed in the environment in which it is employed, ambient light rejection techniques need to be developed and validated with these working standards for maximum effectiveness. Nonetheless these disadvantages are outweighed by the stability of the solid QDot based standard as compared to organic fluorescent dyes that undergo significant photodecay upon illumination with excitation light. Another advantage of the QDots based luminescent working standard is that it could be used as a standard for other biomedical fluorescence spectroscopy applications at other wavelengths besides NIR to create a standard for systems that employ visibly excited dyes and autofluorescence. There are ongoing research activities at national metrology institutes for candidate materials to use as solid fluorescence standards that can serve a broad range of fluorescence instrumentation. These include metal ion-doped glasses [18] and dye-doped polymers [19] but these are still in investigation phase. It should be noted that these reference materials do not have radiometric units but are rather designed for use as relative spectral intensity correction reference.

V. CONCLUSIONS

Herein, we employ a stable phantom to serve as a working standard with constant and known radiance in SI units for comparison of overall imaging device performance. From measurements with the working standard, both ICCD and IsCMOS based imaging devices performances were assessed in terms of contrast, SNR, and resolution at excitation irradiance levels typical of human studies of non-invasive lymphatic function. The working standard demonstrates how overall device performance can be altered with change of detector and operating conditions. We propose that this or a similar working standard be adopted to assess performance using traceable measurements.

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Fig. 1.

Setup for calibrating and measuring the radiance of QDots 800 based luminescent solid phantom and comparing performance of ICCD and IsCMOS NIRF imaging systems. (a) Collection of light exiting the output aperture of an integrating sphere illuminated by an 830 nm laser source via an optical fiber. (b) Detection of the emitted signals from QDots 800 based luminescent solid phantom excited by a 785 nm laser source. (c) Evaluation of measurement performance of ICCD and IsCMOS based NIRF imaging systems using QDots 800 based luminescent solid phantom. The angle of illumination was exaggerated for illustration purposes only.

Fig. 2. Illustration of the radiative transfer and quantities pertinent in this calibration. A are the relevant optical areas, L are radiances, E is irradiance and ω are the solid angles.

Fig. 3. Setup for assessing the resolution of NIRF imaging system using a negative USAF 1951 standard resolution target placed at the output aperture of an integrating sphere illuminated by a 830 laser source via an optical fiber.

Fig. 4.

(a) Calibration curve of mean counts of ICCD camera from the exit aperture image versus the radiance of 830 nm laser diode. (b) Emission radiance at 830 nm per 785 nm excitation irradiance at different concentrations of QDots 800 based solid phantom.

Fig. 5. Calibration curve of mean counts of ICCD camera over the exiting aperture versus the lower (a) and higher (b) radiance of 830 nm laser diode, demonstrating the dark noise of the detector and the maximum measurable radiance.

Fig. 6.

(a) NIRF images acquired using ICCD and IsCMOS camera systems at various setting (full frame, binning mode, integrating times and the gain of the intensifier). (b) The ICCD and IsCMOS average emission intensity as a function of radiance in SI unit measured at the object focal plane for various camera settings.

Fig. 7.

The plots of signal-to-noise ratio (a, b) and contrast (c, d) as a function of radiance in SI unites measured at the object focal plane for various camera (full frame, binning mode, integrating times and the gain of intensifier), demonstrating the difference performance of ICCD and IsCMOS based NIRF imaging systems.

Fig. 8. The acquired images of a negative USAF 1951 standard resolution target (a) and (b) at full frame by ICCD and IsCMOS, respectively, and (c) and (d) at binning 2 and 4 by ICCD and IsCMOS, respectively. The field of view for all views was 10.0 cm in diameter.

Fig. 9. The horizontal (a) and vertical (b) contrast transfer functions (CTFs) as a function of spatial resolution of ICCD based NIRF imaging system operating at full frame.

Table 1

The transverse and lateral resolutions of ICCD and IsCMOS based NIRF imaging systems operating at full frame and binning modes.

	ICCD		IsCMOS	
	Transverse (μm)	Lateral (μm)	Lateral (μm)	Lateral (μm)
Full frame	323	362	182	194
Binning 2	400	417	-	-
Binning 4	-	-	291	263