Comparison of High Sensitivity BLI Imaging Systems for Ultra-weak Signal Applications

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Abstract

Bioluminescent Imaging (BLI) has become a standard laboratory tool that is particularly useful because of its high sensitivity and low backgrounds. It is used for demanding applications such as the location of small tumor metastatic lesions and rapid determination of the efficacy of an intracardiac (IC) or tail vein injection. For IC injections, images taken a few minutes after injection can show whether the procedure was successful (Drake et al., 2005).

All of these applications require measurements near the threshold of detection for even the highest sensitivity imaging systems. Figure 1 shows four mice with IC injections of 1 x 10^5 22Rv1.lucPN2 prostate cancer cells. Successful IC injection results in systemic cell distribution, which will fall below the limit of detection. In contrast, unsuccessful IC injection results in detectable signal in the cardiothoracic region (Drake et al., 2005). In Figure 1, the two mice on the right underwent successful IC injections as evidenced by the lack of localized signal. The two mice on the left depict unsuccessful IC injections due to the detection of luminescent emission in the cardiac region. These signals are "ultra-weak" and near the limit of detection, with average radiance of a few thousand photons/second/cm2/sr. Thus it is necessary to use a system with a detection threshold significantly below this and to understand any issues which may affect the ability to make these measurements.

For very low signal BLI images taken with a popular high sensitivity system, an IVIS® 100 imaging system, a "halo" consisting of high background in a circular pattern around the center of the image is routinely observed. An example of this effect is seen in Figure 1. In an effort to understand this problem and how it affects quantitation and detection thresholds, similar ultral weak images were studied using IVIS® 200 (now known as the IVIS® Spectrum) and Spectral Instruments Imaging Ami X imaging systems. Measurements were made with a low level calibration light source at the center and near the corners of each of all three systems. It was observed that corner measurements had much higher levels of statistical noise on the IVIS® 100. This was determined to be an artifact of the flat field correction algorithm applied to compensate for the lower offl axis light collection efficiency of the lens. Since the number of photons detected is decreasing moving away from the center of the lens, a correction must be applied to the image to provide a uniform light detection across the field of view (FOV), allowing for correct quantitation. The background statistical noise is relatively flat across the FOV but when the correction is applied, the noise is effectively amplified resulting in the halo effect shown in Figure 1. The degree of this effect is thus strongly dependent on the size of the flat field correction factor. Corner and center measurements were taken on all three systems both with and without flat fielding correction allowing for determination of the correction factor for each system.

The IVIS® 100 correction factor for the measurement near the corner of the image was determined to be a factor of 4.9. The other systems have smaller flat field corrections, 1.6 for the IVIS® 200/Spectrum and 1.5 for the Ami X. The halo effect was not observed on either of these systems.

During the course of this work, the lower limits of these three systems for observation and quantitation of a weak BLI signal was examined. For the region of the image away from the halo effect, all three imaging system lower limits were similar and capable of quantitating ultra-weak BLI signals such as those shown in Figure 1. Near the corners of all three systems, the signal/noise for a low level signal was lower in the IVIS® 100, making it more difficult to detect a low level signal. For the other systems this effect was negligible and they were able to detect ultral weak signals across the entire field of view.

Drake JM ,Gabriel CL, and MD Henry. Assessing tumor growth and distribution in a model of prostate cancer metastasis using bioluminescence imaging. Clin Exp Metastasis. 22:674-684. 2005. PMID: 16703413

Halo Effect in High Sensitivity BLI Image

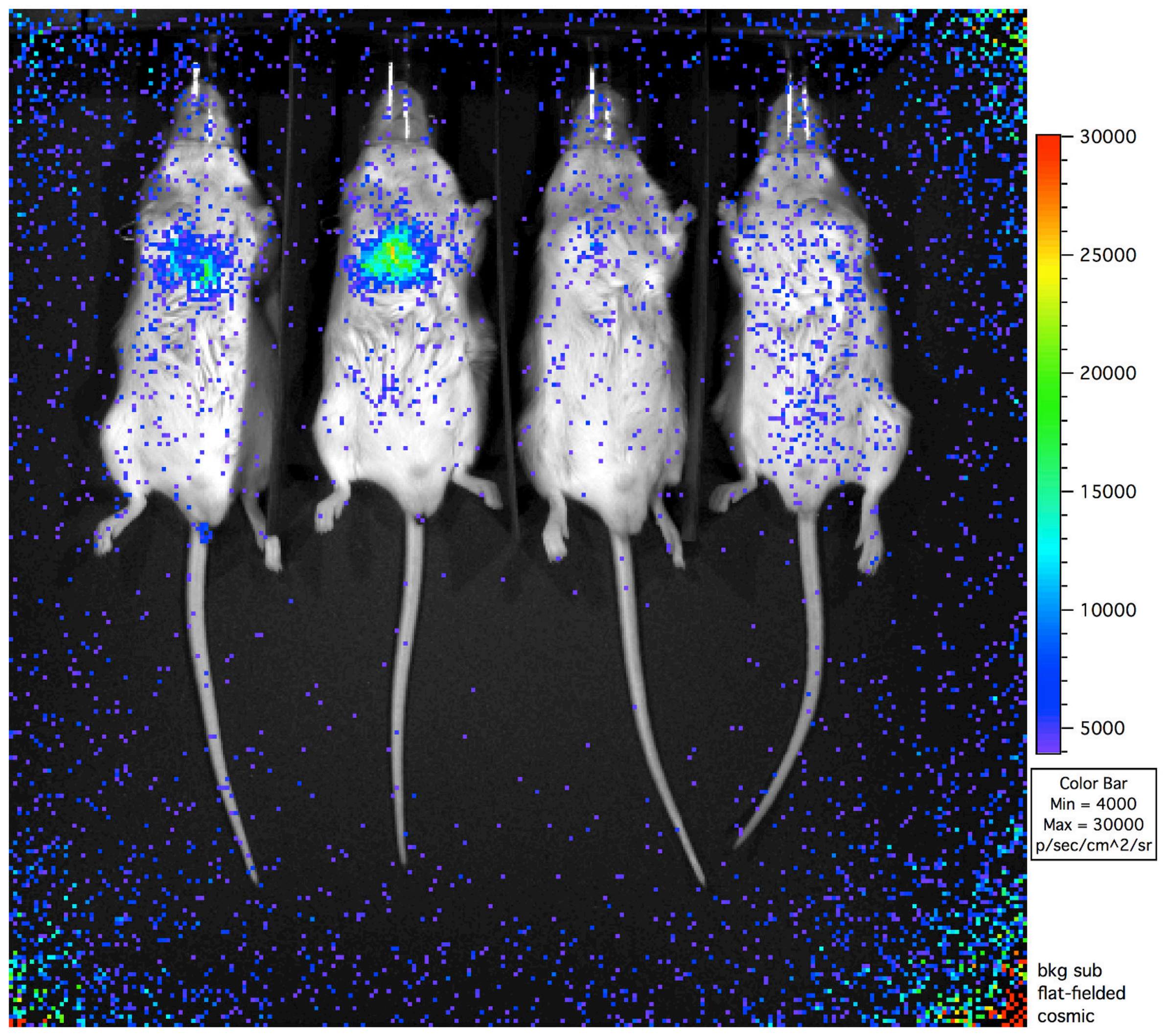

Figure 1: High sensitivity BLI image taken with an IVIS® 100 system. 1 x 10^5 22Rv1.lucPN2 prostate cancer cells were injected intracardiacally into scid mice. Following IC 22Rv1.lucPN2 delivery mice were injected intraperitoneally with Dl lucifern and BLI was performed. The two mice on the left represent unsuccessful IC injections as demonstrated by accumulation of luciferase signal in the cardiothoracic region. The two mice on the right demonstrate successful IC injection with systemic distribution of the cancer cells and luciferase signal below the limit of detection. A highl background "halo" effect is seen as a circular pattern of noisy, high level regions where there is no real signal.

Noise Analysis

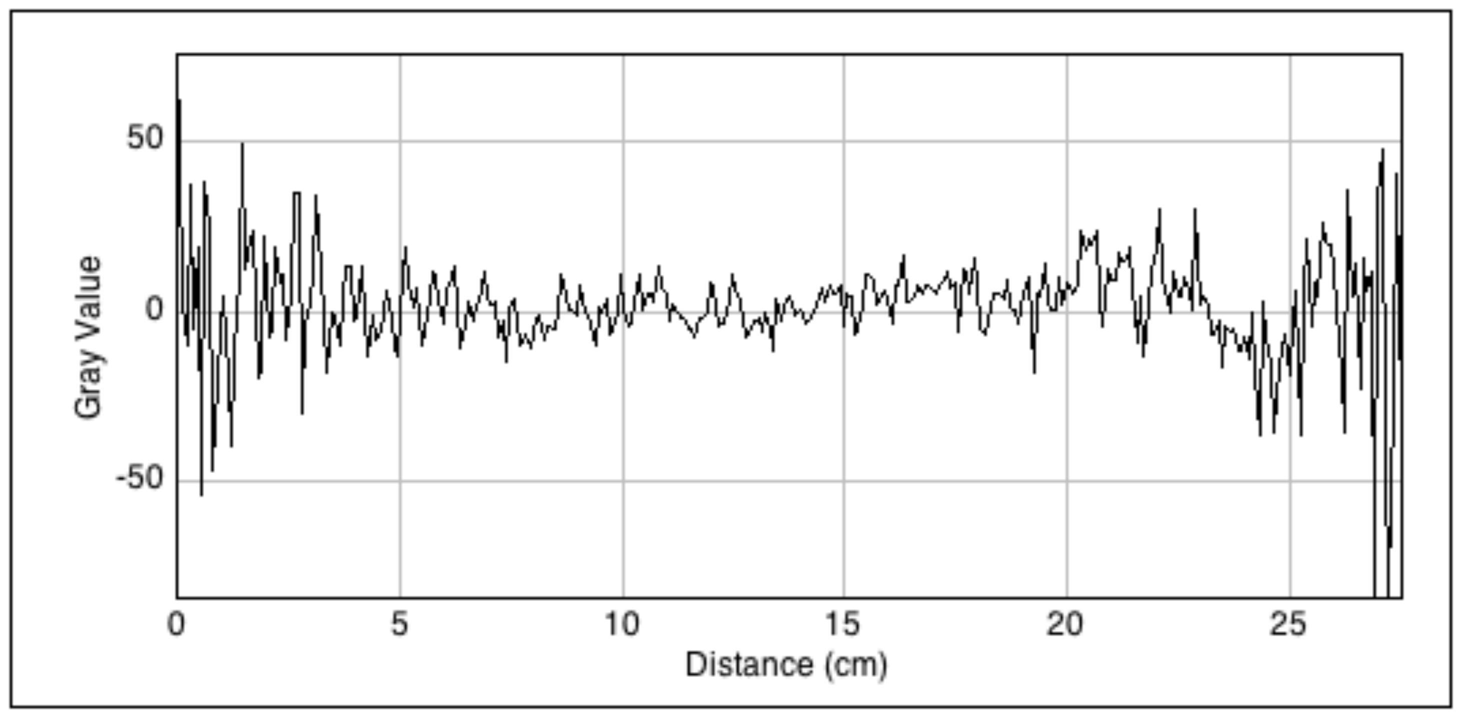

Figure 2: A line profile was used to measure the luminescent signal in Figure 1 along a diagonal path from the lower left to the upper right corners. There is very little real signal along this path with an average value of zero. However, the statistical variation about zero increases greatly in the corners near the ends of the path where the halo signal is most pronounced.

Corner vs. Center Measurements

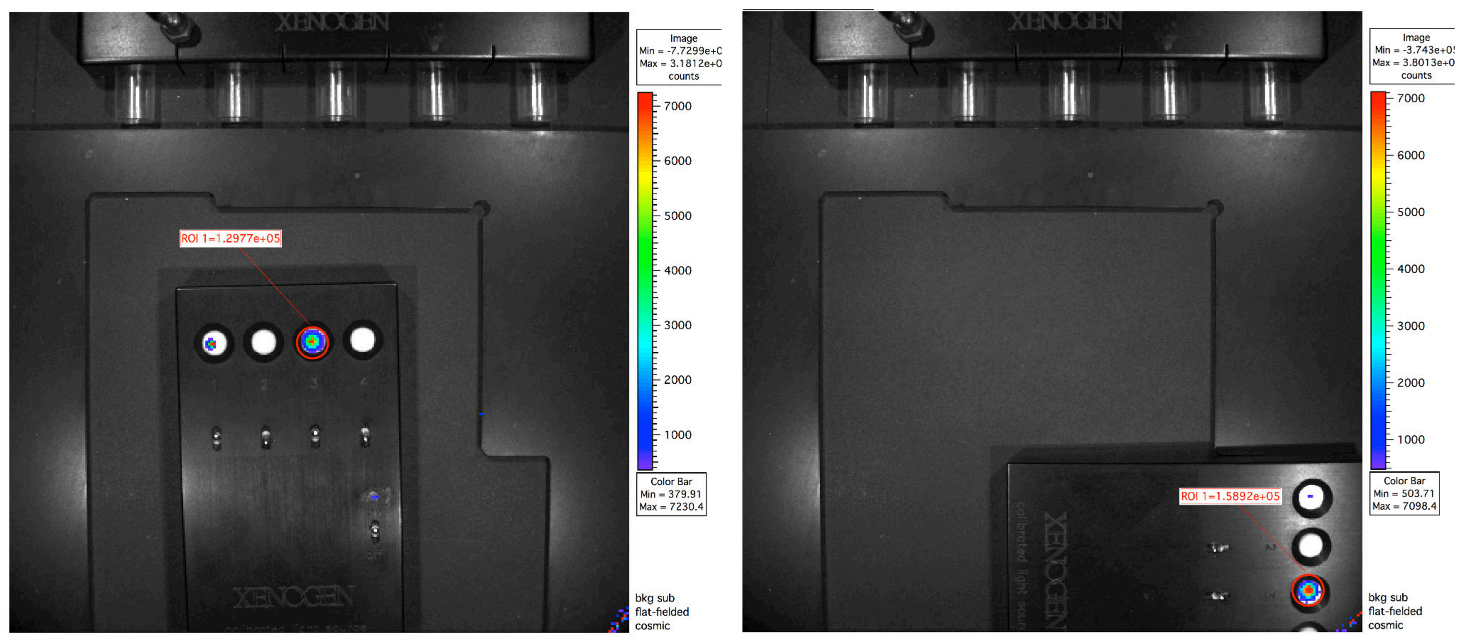

Figure 3: A calibrated light source was used to make Region of Interest (ROI) measurements in the center and lower right corner of the image area. Similar measurements were made on two other high sensitivity BLI systems for comparison. For each system, measurements were made with flat fielding corrections both on and off. The results of these measurements are shown in Table 1.

Center vs. Corner Measurements for Three High Sensitivity BLI Systems

	corner	center	ratio	FF
IVIS®100	1.59E+05	1.30E+05	0.82	on
	2.63E+04	1.29E+05	4.90	off
IVIS®200	4.00E+05	3.83E+05	0.96	on
	2.33E+05	3.80E+05	1.63	off
Ami X	2.89E+05	2.78E+05	0.96	on
	1.83E+05	2.78E+05	1.52	off

Table 1: ROI measurements of a calibrated light source were made in the center and corner of the imaging area for three high sensitivity BLI systems (see Figure 3). For each system, the measurements were made with flat fielding (FF) both on and off. Flat fielding corrections are a multiplier applied in software to adjust signal levels for decreasing light collection efficiency in areas of the image away from the center. The magnitude of flat field correction required for a system is a property of the light collection optics. The column labeled "ratio" is the ratio of the center to corner signal and is close to one when the flat field correction is applied. When flat field is off, the observed ratio is the value of the correction required to provide uniform measurement values across the image area. It is seen here that the IVIS® 100 system requires a much larger flat field correction than the other two systems. This large correction introduces increased statistical noise away from the center of the image and explains the profile observed in Figure 2.

Photon Measurements for Three High Sensitivity BLI Systems

	photons/s	error
Calibrated Source	2.84E+06	---
IVIS®100	2.62E+06	7.7%
IVIS®200	2.03E+06	28.5%
Ami X	2.59E+06	8.8%

Table 2: ROI measurements of the calibration light source made for Table 1 are compared in photons units. All measurements are within 30% of the source value for the three systems. This quantitative agreement allows comparison of measurements among these and other calibrated BLI systems.

High Sensitivity BLI Images Comparison

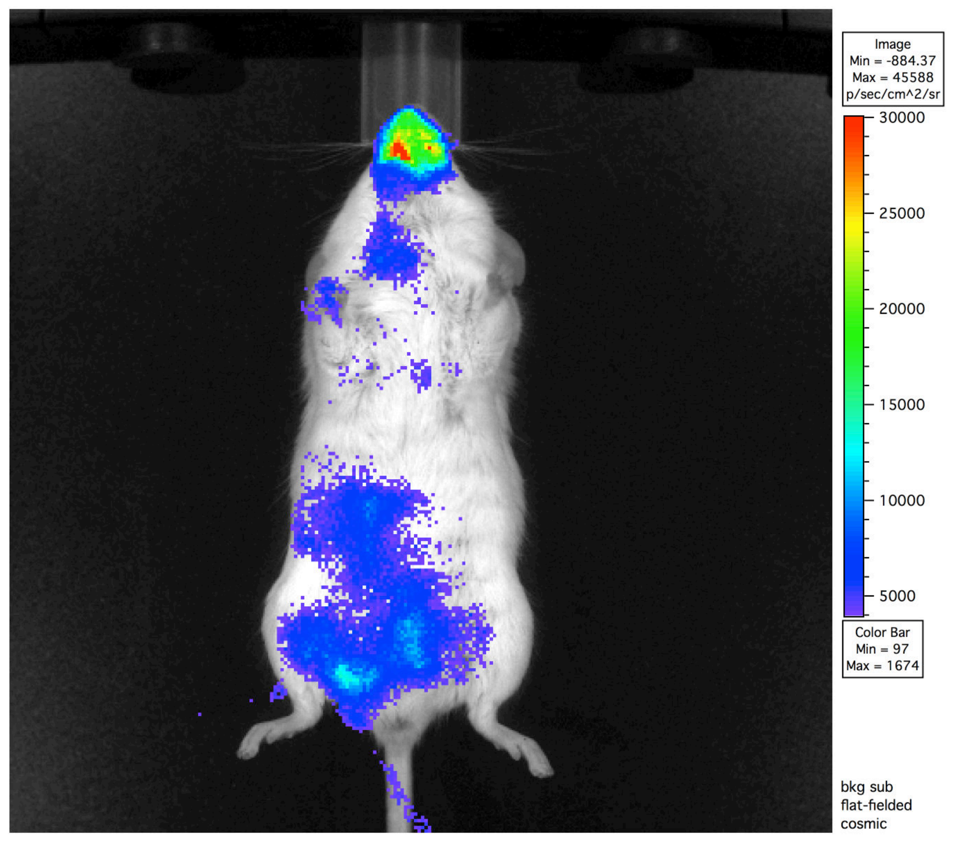

Figure 4: High sensitivity BLI image taken with an IVIS® 200 system. A C57BL/6 LSLI luciferase TP53$^{fl/fl}$ PTEN$^{fl/fl}$ mouse was exposed to adenovirus containing a Cre recombinase expression vector. The virus was introduced by topical application to the anterior dorsal tongue to promote luciferase expression and TP53/PTEN loss. BLI was obtained 16 weeks postl viral exposure following intraperitoneal injection of Dl lucifern substrate. Photon sensitivity range is set identically to Figure 1. As expected from the manufacturer's specification, this system is as sensitive as the IVIS® 100 but does not exhibit the halo effect.

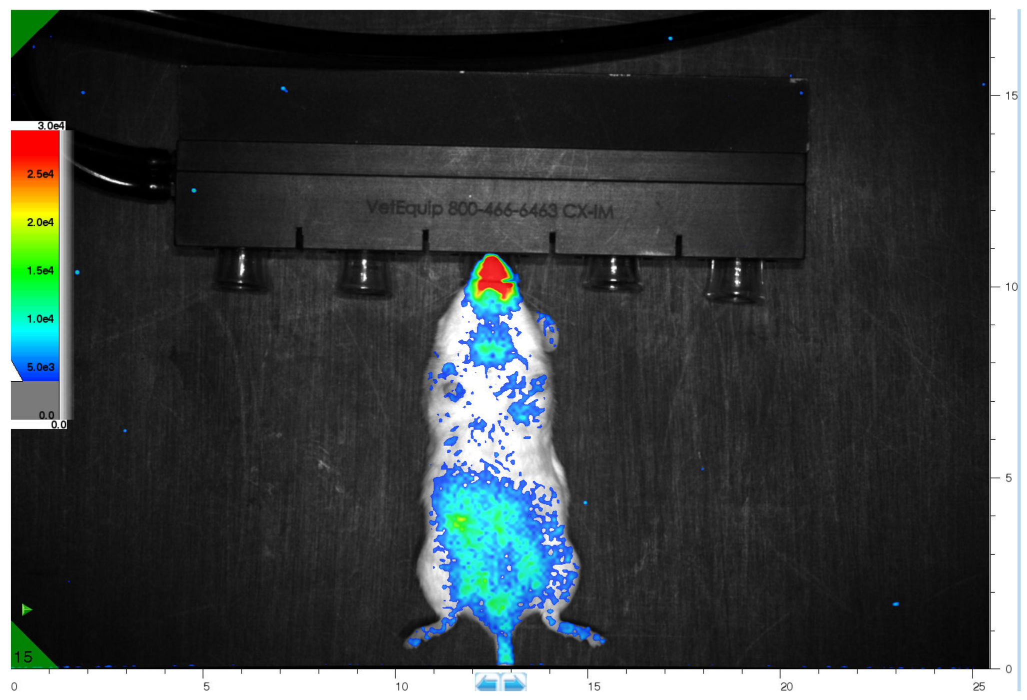

Figure 5: High sensitivity BLI image taken with an Ami X system. The same C57BL/6 LSL-luciferase TP53$^{fl/fl}$ PTEN$^{fl/fl}$ mouse 16 weeks post-viral ex posure wa s im aged fo llowing D-luciferin intraperitoneal injection. Photon sensitivity range is set identically to Figure 1. As expected from the manufacturer's specification, this system is as sensitive as the IVIS® 100 but does not exhibit the halo effect.

Conclusions

- **IVIS® 100 halo effect in weak image is due to a software correction applied to correct for lower off-axis lens efficiency**
- **All three instruments achieve color range required for high sensitivity BLI imaging, but IVIS® 200 and Ami X lenses require less correction resulting in no halo**
- **All three instruments measure same photons level of reference source, so quantitative comparisons can be made among systems**