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 10⁶ 22Rv1.lucPN2 prostate cancer cells. Successful IC injection results in systemic cell distribution, while 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 radian of a few thousand photons/second/cm²/sr. Thus it is necessary to use a system with a detection threshold significantly below this 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 ultra-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 off-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 field 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 49. 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 ultra-weak signals across the entire field of view.

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.

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