# Automated, Fast, High-Sensitivity Optical Imaging with the Spectral Instruments Imaging Lago X

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## Abstract

**To simplify the logistics of high-volume preclinical optical imaging, it is important that it be both easy and fast. Spectral Instruments Imaging (SII) has realized these two criteria by further enhancing their imaging software platform, Aura, with new, powerful features: (1) automated Easy Mode imaging, powered by Smart Auto Exposure (SAE), a proprietary matrix of imaging algorithms, and (2) Smooth Blend, which causes all optical signals to be presented with a smooth outer-edge profile (without any change in signal strength) and so enables fast imaging to be done and presented without signal pixelation, which may obscure in vivo signal location within animal models.**

**In this poster, we present optical imaging data confirming two critical points: (1) the automated SAE algorithms of Easy Mode produce accurate optical signal radiance values (where radiance data acquired by standard, manual imaging protocols are used as the gold standard) and (2) Smooth Blend does not alter optical signal values (where region of interest (ROI) analyses of Classic View data served as the gold standard).**

**Beyond such proof-of-concept data sets, we also demonstrate here, with various bioluminescent, osteosarcoma mouse models, that the SII Lago X imager can out-perform the Perkin Elmer® (PE) IVIS® Spectrum on the basis of superior signal sensitivity. In a side-by-side comparison, sets of mice were sequentially imaged in an IVIS® Spectrum and then in a SII Lago X, at peak bioluminescent signal post-luciferin injection, and ROI Total Emission values (photon/sec) of the SII Lago X were regularly greater than those reported by the IVIS® Spectrum.**

## Methods & Results

### Equivalent optical signal values are observed when imaging by either Automatic Easy Mode with Smart Auto Exposure algorithms or by Standard manual settings.

SII Aura software ***Easy Mode*** imaging, empowered by a proprietary set of imaging algorithms known as ***Smart Auto Exposure (SAE)***, has successfully simplified the optical imaging experience into a 1-2-3 process. An investigator need only select an imaging **Subject** type (e.g., in vivo mouse, in vivo rat, etc.), and an optical **Probe** (bioluminescent or fluorescent), and then they can start imaging by hitting the **Acquire** button (see **Figure 1**).

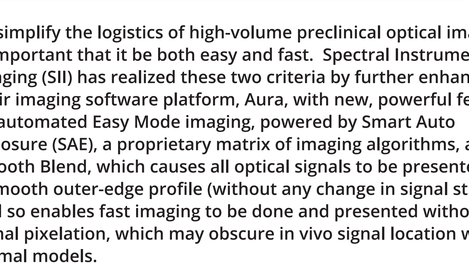


***Figure 1: SII Aura Easy Mode.***

Given the simple convenience of Easy Mode, it was imperative to test that Easy Mode produces the same, accurate optical imaging results achieved by Standard manual settings.

To this end, a series of controlled light sources were set up and imaged by both Easy Mode and Standard manual protocols, and the resulting ROI Total Emission (photons/sec) results were compared. An SII Calibration Device (SIICD) was used as a baseline, controlled light source. It is equipped with two visible light sources, with the light source to the right being weaker. In the experiment presented here, to develop a series of controlled light sources the following steps were taken: (1) the weaker light source of the SIICD was completely blocked by a section of black paper, and (2) the stronger light source was sequentially imaged as it was being attenuated by a series of Neutral Density (ND) filters, where the ND filters ranged from 0.2 ND to 3.0 ND. Note: ND filters are known to equally block all wavelengths of visible light, and the amount of light blocked correlates positively with the ND filter number. For each ND filter set-up tested, 5 replicate images were acquired by Easy Mode and then by Standard manual imaging protocols (see **Figure 2**). Resulting ROI Total Emission evaluations were then plotted vs. ND filter used. In summary, for all ND filters tested (0.2 to 3.0 ND), it was observed that automatic Easy Mode, SAE-powered and Standard manual imaging protocols produced highly similar optical signal results, with % differences in Total Emission mean values (photons/second) ranged from 4.6 % down to just 0.70 % (see **Figure 3**).

***Figure 2: Test images taken to determine if light signal values are highly conserved between Easy Mode SAE algorithm and Standard manual setting acquisitions.***

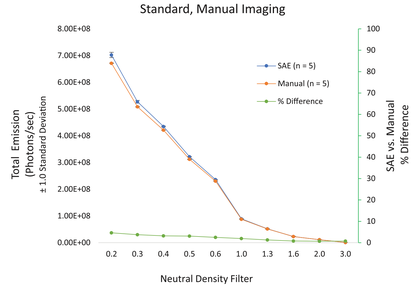


***Figure 3: Light signal values were highly conserved between Easy Mode SAE algorithm and Standard manual setting acquisitions.***

### Equivalent optical signal values observed when conducting analyses under either Smooth Blend or Classic View settings.

Use of the **Smooth Blend** feature causes all optical imaging signals to have a smooth, outer-edge profile. A smooth signal profile will eliminate signal pixelation and optimize signal esthetics. In turn, the use of Smooth Blend is meant to encourage the use of high-sensitivity settings that will enhance speed and increase signal detection. Of course, to use Smooth Blending, it is imperative that the feature has no quantitative effect on reported optical signals. To demonstrate that Smooth Blending meets this performance criterion, a set of images was acquired and then put through a ROI Total Emission analysis with or without the activation of Smooth Blending.

To conduct a rigorous version of such a test, we sequentially imaged a SII calibration device (SIICD, a controlled, constant light source) under 5 distinct camera settings, with each using a different bin-level setting (1x1, 2x2, 4x4, 8x8, or 16x16, see **Figure 4a and 4b**). Ten replicates of these 5-image sets were acquired. The stronger light source of the SIICD was used as an imaging target. Given that Smooth Blend is a post-acquisition, Aura was set to use either Classic View (no filter) or Smooth Blend prior to running ROI Total Emission analyses. For any given bin level, across the 10 replicate images acquired, it was observed that ROI Total Emission value means, run under either Classic View or Smooth Blend settings, were nearly identical, differing only by 0.03-0.04 % (**Figure 4c**).

Finally, Smooth Blending's neutral effect on ROI Total Emission analyses was also evaluated for a single image of ten mice, where each mouse was challenged by an ectopic bioluminescent tumor mass on their right thigh (see **Figure 4d**). In this less rigorous evaluation (i.e., where no multiple-image, variable camera settings were used), ROI Total Emission analyses under Smooth Blend and Classic View settings showed complete identity (unity) across all mouse bioluminescent signals evaluated (see **Figure 4e**, $R^2 = 1$).

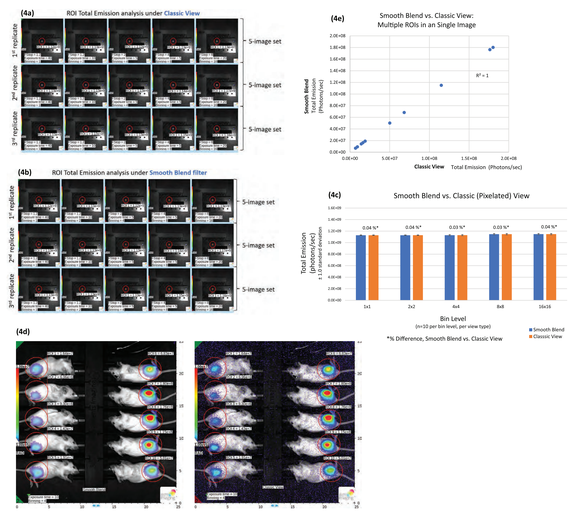


***Figure 4: Test images taken and analyzed to determine if Smooth Blend filter has any effect on light signal values***

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### SII Lago X optical imaging by either automated Easy Mode SAE or Standard manual settings appeared to be more sensitive than PE IVIS® Spectrum using an Auto Exposure (AE) protocol.

The combined introduction of Easy Mode imaging, powered by SAE, and Smooth Blend has made in vivo optical imaging respectively easier and faster. It remains to be demonstrated, however, if the use of these imaging features in a SII Lago X will lead to an optical imaging performance that is more sensitive than other competing systems such as the IVIS® Spectrum from Perkin Elmer® (PE). To address this point, various bioluminescent osteosarcoma mouse models were imaged sequentially, in both an IVIS® Spectrum and a Lago X, during a previously established period of peak bioluminescence, at 10-20 minutes post-luciferin injection.

**Analyzing Living Image® (LI) Files in SII Aura Software.** Now, before presenting the above-mentioned, comparative image analyses, it should be noted here that all such comparative optical signal evaluations, of both IVIS® Spectrum and SII Lago X images, were conducted in SII's software platform, Aura. This was viewed as an efficient and transparent analytical approach on the basis of the following three facts: (1) IVIS® Spectrum images acquired by Living Image® (LI) software can be loaded and analyze in SII Aura software, (2) IVIS® Spectrum image signals put through replicate ROI analyses, in LI and Aura lead to identical Total Emission results (see **Figure 5a and 5b**), and finally (3) by using a single "rainbow" look-up table in Aura software, the relative radiance (p/s/cm²/sr) of IVIS® and SII optical signal data could be accurately and transparently presented.

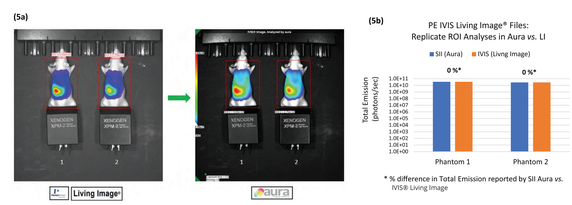


***Figure 5: Analyzing Living Image® (LI) Files in SII Aura Software***

**Intrafemorally (IF) challenged mice in an osteosarcoma model:** It was observed, across several time points, that Easy Mode SAE and Standard manual bioluminescent imaging in the Lago X was notably more sensitive than Auto Exposure (AE) imaging in the IVIS® Spectrum, as determined by replicate ROI Total Emission analyses. Example comparison data of SII Lago X imaging (under Easy Mode SAE or Standard manual settings) vs. IVIS® Spectrum AE imaging are illustrated in Figures 6a/c, and quantitatively summarized in Figures 6b/d. It was also observed in the above comparisons that over identical dynamic ranges of signal presentation (note identical calibration bars in relevant figures), Lago X image files showed an optical signal in mouse #1, while IVIS® Spectrum image files did not (see blue arrows in Figure **6a and 6c**).

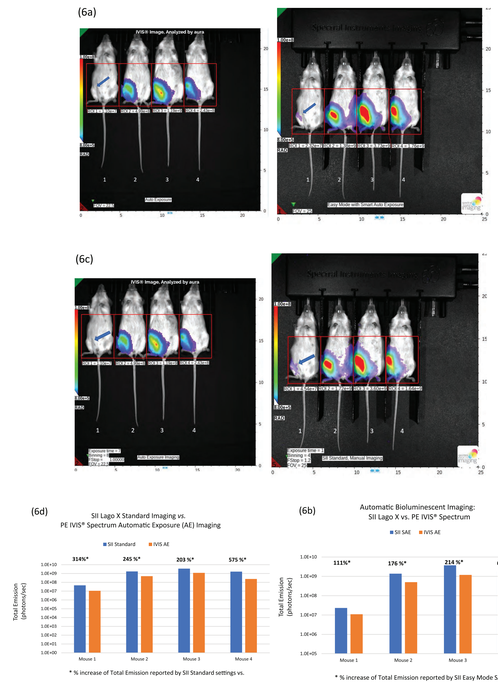


***Figure 6: Intrafemorally (IF) challenged mice in an osteosarcoma model***

**Intravenously (IV) challenged mice in an osteosarcoma model:** Again, it was observed, across several time points, that Easy Mode SAE bioluminescent imaging in the Lago X was notably more sensitive than Auto Exposure (AE) imaging in the IVIS® Spectrum, as determined by replicate ROI Total Emission analyses. An example comparison of SII Lago X Easy Mode SAE imaging vs. IVIS® Spectrum AE imaging is presented in Figures **7a/b/c,** and quantitatively summarized in Figures **7d.** Interestingly, in this model, Lago X Easy Mode SAE imaging automatically presented an optical signal in both Mouse #1 and #2, while IVIS® Spectrum AE imaging only showed a signal in Mouse #2. Indeed, it was only after Mouse #2 was removed from the field of view (FOV) that the IVIS® Spectrum AE imaging was able to automatically detect and present any signal from Mouse #1 (see **Figure 7b/c**).

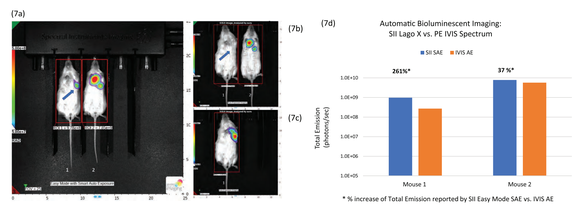


***Figure 7: Intravenously (IV) challenged mice in an osteosarcoma model***

## Conclusions

**The detection of controlled, bioluminescent signals by automated Easy Mode Smart Auto Exposure algorithms and Standard manual camera setting protocols were observed to be tightly correlated by ROI Total Emission (photon/sec) analyses.**

**SII Aura's Smooth Blend feature was observed to have essentially no effect on the reporting of bioluminescent signal data by ROI Total Emission (photon/second) analyses.**

**Optical imaging analyses of several bioluminescent osteosarcoma mouse models, performed by both a SII Lago X and a IVIS® Spectrum (in a side-by-side set up), clearly demonstrated that the Lago X, operating either under Easy Mode Smart Auto Exposure algorithms or Standard manual settings, was consistently more sensitive than the IVIS® Spectrum using its AE protocol.**