Kinetics Software Technology from Spectral Instruments Imaging Dramatically Improves how *In Vivo* Bioluminescent Data is Acquired and Quantified

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Introduction

In any bioluminescent (BL) animal model, where luciferase substrate is injected, the amount of BL signal produced is *not* constant. Rather, it has been well established that BL signal flux (total photons/sec) will vary as a function of luciferase substrate *in vivo* pharmacokinetics. Typically, in a graph of BL signal vs. time post-substrate injection, BL signal flux will sequentially rise, plateau (peak), and fall over time, and correlation between BL signal flux and time post-substrate injection is referred to as the BL kinetic curve of the model (Figure 1). It has been determined that various additional factors can also affect BL kinetic curve profiles. A summary of these identified additional factors is presented in Figure 2A and 2B.

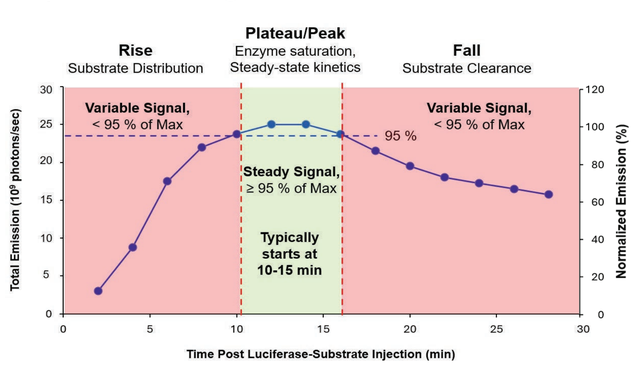
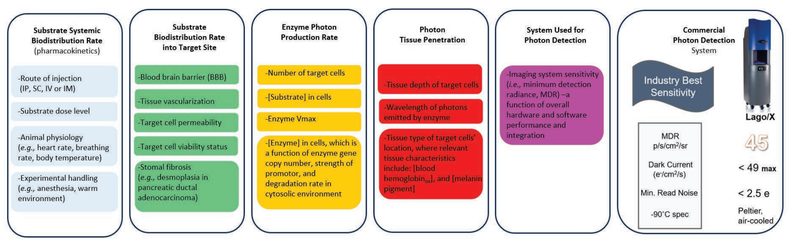


Figure 1: Phases of a BL Kinetic Curve. In a typical BL kinetic curve, BL signal data will go through three phases—rise, plateau/peak, and fall—as a function of time post-luciferase-substrate injection.

It is widely recognized that in preclinical BLI animal models one should report only plateau-phase or *peak BL data* (Sim, et. al., 2011), as such BL values will optimize BL model sensitivity, reproducibility, and biological relevance—only peak BL data is produced under steady-state, saturation kinetics, where the amount of BL signal correlates tightly with the amount of luciferase enzyme present in the animal model. Therefore, peak BL signal is optimally and consistently correlated with a given animal model's number of BL cells.

With the objective to automate and to easily acquire peak BL data in real-time, Spectral Instruments Imaging (Spectral) has developed *Kinetics* mode, as a new feature in *Aura 4.5* software. *Kinetics* has been designed to acquire and to present the BL kinetic curve data of up to 10 mice from a single image. To demonstrate the current performance capabilities of *Kinetics*, several "proof-of-concept" (POC) studies were conducted first with light phantoms and then with live-mouse oncology models. The observed performance of *Kinetics* in these POC studies are summarized and presented here, in this poster.



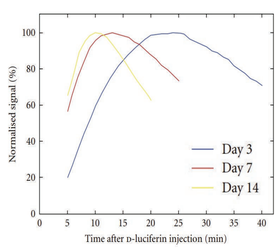


Figure 2a (top): Range of factors that can affect BL kinetic curve profile. Beyond substrate pharmacokinetics, BL kinetic curve profiles are known to be affected by the above presented factors (adapted from Kim, M., et. al., 2022, and Inoue,Y,. et. al., 2010).

Figure 2b (left): BL kinetic curves can vary over the time course of a solid tumor model. As subcutaneous tumors grew over time, plateau peak kinetics were observed to occur earlier and over shorter lengths of time (Inoue, et. al. 2010).

Material & Methods

***Kinetics* Software Feature in Spectral's Aura 4.5.** *Kinetics* mode operates as a feature within Spectral's established optical imaging platform known as *Aura*. The imaging protocol followed in *Kinetics* is outlined in Figure 3. Prior to running a *Kinetics* acquisition, one selects values for several imaging parameters: (i) the number of mice to be imaged (5 or 10) as this guides the number of regions of interest (ROIs), (ii) the total run time of the acquisition, (iii) the interval time between images, and finally, (iv) the time passed since mice were injected with substrate. Once *Kinetics* is launched, it will automatically present images as they are acquired, and perform whole-mouse, BL signal ROI analyses (Total photon/sec). Individual and group mean BL kinetic curves are derived and presented in a live graph, in real time, where Total photons/sec is plotted vs. Time post-substrate injection. Finally, an exportable summary table of BL data is also provided. Using the graph and table together, exact peak BL values can be readily identified.

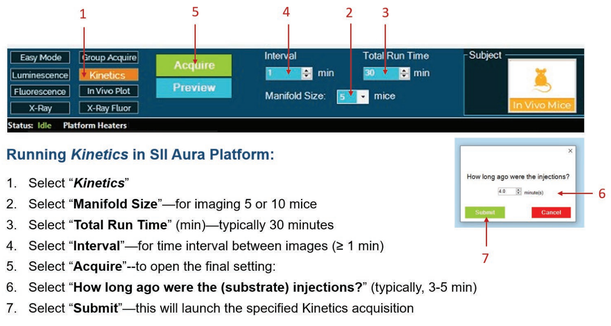


*Figure 3: The protocol for running **Kinetics** Feature in Spectral's **Aura** Software Platform*
*A **Kinetics** imaging session can be launched easily in seconds—One simply needs to select a few imaging parameter options, from the set of pull-down menus illustrated above.*

***Kinetics* Imaging of Light Phantoms.** In an initial POC study, *Kinetics* was evaluated on its ability to acquire and present BL kinetic data from 5 programmed light phantom targets. The flux rates of these phantoms (total photons/sec) were programmed to mimic the rise, plateau/peak, and fall sequence typical of *in vivo* BL kinetic curves.

***Kinetics* Imaging of Mouse Oncology Models.** *Kinetics*' ability to acquire and present BL kinetic data from live mice was tested in a 10-mouse experiment of two oncology models. B-cell leukemia model mice were 4-week old, female NSG mice challenged with Nalm6 cells by IV injection, while the myeloma model mice were 4-week old, female NSG mice challenged with MM1S cells by IV injection. In both models, the BL cell lines were transduced to express Firefly luciferase. Both sets of B-cell leukemia model mice (n=5) and myeloma model mice (n=10) were concurrently imaged, side-by-side, in a 25 cm x 25 cm field of view in a Spectral Lago X *in vivo* imaging system.

Animal Handling Protocol for Kinetics Imaging. Just prior to imaging, mice were subcutaneously (sc) injected with D-luciferin substrate and then anesthetized in the presence of isoflurane vapor. Specifically, test mice received a 100 µL dose of 30 mg/mL D-luciferin (i.e., a 150 mg/kg dose, given that mice were all ~20 g), by sc injection (along the right flank), and were then anesthetized in an induction chamber, by exposure to 2.5% isoflurane vapor, delivered at 2 L/min. During imaging, all mice remained anesthetized by inhalation of 2.5% isoflurane vapor, delivered at 1 L/min).

Results

Experimental results illustrated that *Kinetics* software was consistently able to acquire and to present the individual and group mean BL kinetic curve data of up to 10 mice at-a-time. This data production was done in a fully automated fashion, and data was presented graphically in real time.

***Kinetics* Imaging Light Phantoms.** In the POC phantom imaging experiment, BL signal values were acquired at 1-min intervals, over a span of 30 min, using a 5-mouse manifold format. Individual and group mean ROI BL values were automatically acquired and then used to produce both a real-time graph of BL kinetic curves, and an exportable, summary table of BL ROI data (Figure 4A and 4B).

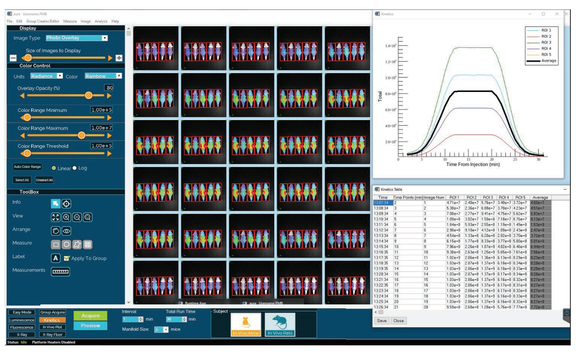


*Figure 4a: BL Phantom "Proof-of-Concept" (POC) Study: BL data sets produced by **Kinetics**. A set of 30-images were automatically acquired, put through an ROI analysis, and then used to propagate (i) a live graph of Total (Emission, Total photons/sec vs. Time post-substrate injection), and (ii) a summary table of acquired image data, listing: (a) image acquisition times, image time points (min post-substrate injection), (c) image number, (d) whole phantom ROI Total (Emission, Total photons/sec) individual and mean values.*

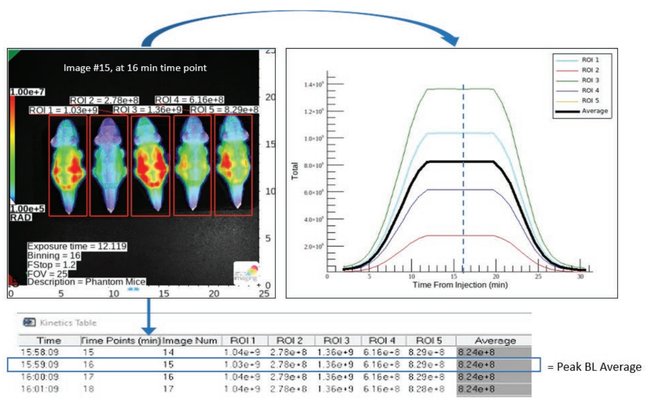


Figure 4b: Phantom POC Study: ROI BL image data supports a live graph, in real time, and populates a summary data table. ROI BL signal in example image (#15), was taken at 16 min from start of phantom light program. Automatically acquired ROI BL data was propagated in real-time from image #15 to points in the live graph and summary table (see blue arrows).

***Kinetics* Imaging of Mouse Oncology Models.** In the *Kinetics* POC study with 10 live mice from 2 BL oncology studies (B-cell leukemia and myeloma, see M&M), mice were initially imaged on day-7 post-challenge, at 1-min intervals, over a span of 30 min, using a 10-mouse manifold format. From the images acquired, individual and group mean ROI BL values were attained and then used to produce: (i) two real-time graphs (of mice #1-5 and #6-10, separately), illustrating of individual and group mean BL kinetic curves, and (ii) a single summary, exportable table of ROI BL data (Figure 5A, 5B and 5C).

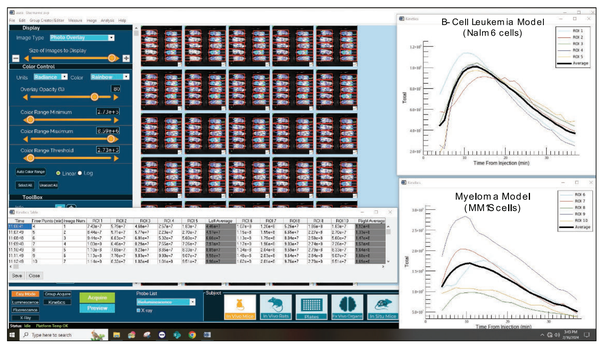


*Figure 5a: B-cell Leukemia and Myeloma "Proof-of-Concept" (POC) live-mouse studies: BL data produced by **Kinetics**. Individual mouse and group mean BL ROI data, from 30 acquired images of two oncology models—mice #1-5 (B-cell leukemia model) and mice #6-10 (a myeloma model)—were presented separately in two, distinct graphs, while BL data of all 10 mice were presented in a single summary table. The two oncology models appeared to have distinct profiles, with different BL peak times post-substrate injection (see Figures 6A and 6B).*

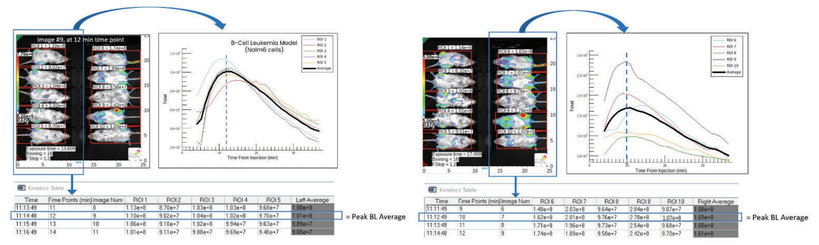


Figure 5b (left): B-cell Leukemia POC Study: ROI BL image data supports a live graph, in real time, and populates a summary data table. An example presented here uses image (#9), taken at 12 min post-substrate injection. Automatically acquired ROI BL data was propagated in real-time from image #9 to points in the live graph and summary table (see blue arrows).

Figure 5c (right): Myeloma POC Study: ROI BL image data supports a live graph, in real time, and populates a summary data table. An example presented here uses image (#7), taken at 10 min post-substrate injection. Automatically acquired ROI BL data was propagated in real time from image #7 to points in the live graph and summary table (see blue arrows).

A comparative analysis of Mouse Oncology Kinetic Curve Profiles. On day-7 post-challenge, *Kinetics* enabled a BL kinetic curve analyses of two oncology models: a B-cell leukemia model (n=5, mice #1-5) and a myeloma model (n=5, mice #6-10) (Figure 5A). The BL kinetic curve profiles of each model were observed to be distinct, both in shape and in the time of peak BL data (Figure 6A). Plateau-phase kinetics (when BL data was ≥ 95% of peak BL value) lasted for about 5.5 min in each model, but the timeframes of plateau-phase kinetics were staggered: 7.5-13 min in the myeloma model, vs. 9-14.5 min in the B-cell leukemia model (Figure 6B).

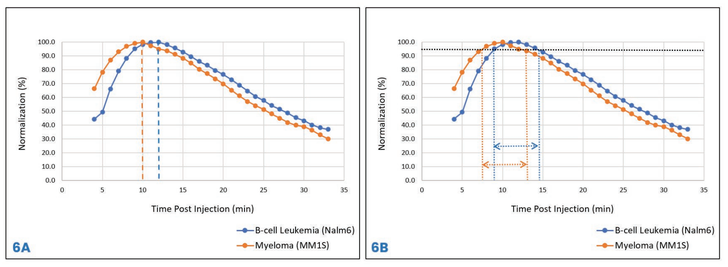


Figure 6: Kinetic Curves of B-cell leukemia and myeloma studies at day-7 post challenge: An analysis of when peak BL and plateau-phase BL data occurred. It was observed that B-cell leukemia and myeloma mice demonstrated: (i) distinct times of peak BL data: at 12 minutes vs. 10 minutes post-substrate injection, respectively (6A), and (ii) distinct periods of plateau-phase kinetics (where BL sigal was ≥ 95% of peak BL value): at 7.5-13 min in the myeloma model, vs. 9-14.5 min in the B-cell leukemia model (6B).

Changing BL kinetics over time course of B-cell Leukemia model. Individual and mean BL kinetic curves of a B-cell leukemia model (see M&M) were determined by *Kinetics* mode at 7-, 14-, 21-, and 27-days post-challenge. It was observed that peak BL data occurred at distinct times post-substrate injection over the time course of the study (Figure 7A-7H). Furthermore, it was noted that the kinetic curve profiles changed dramatically between day 21 and day 27 post-challenge. The relatively long persistence of plateau-phase BL kinetics on day 27 post-challenge would suggest one or more types of physiological change(s) in the mouse model, in the distal and/or immediate environment of the BL leukemia cells (for a review of possible causes to consider, see Figure 2A.)

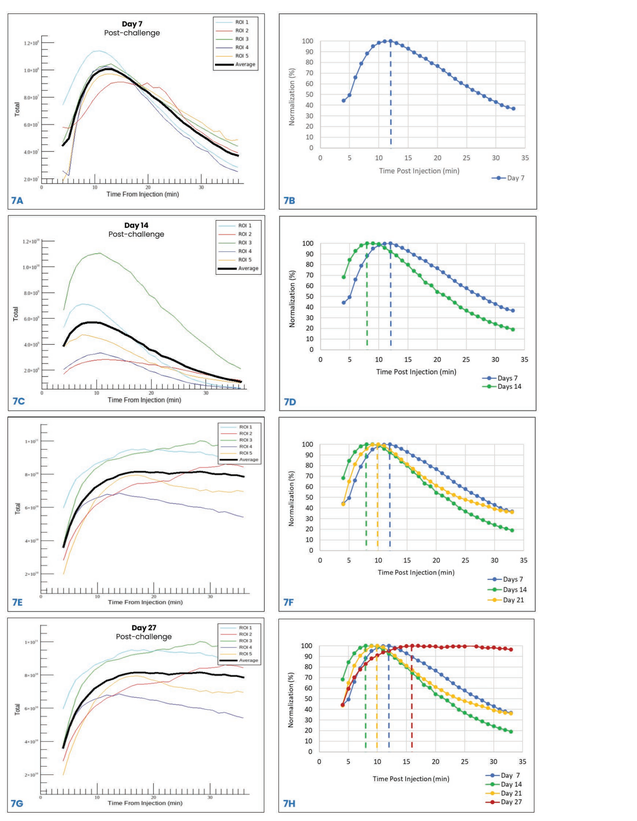


Figure 7: B-cell leukemia model kinetic curves over time course of POC study: An analysis changing group mean BL peak times, and kinetic curve profiles. Group mean peak BL data was observed to occur at distinct times (post-substrate injection) over the time course of the study: at 12 min on day-7 (7A-B), at 8 min on day 14 (7C-D), at 10 min on day 21 (7E-F), and at 16 min on day 27 (7G-H). Furthermore, it was noted that the graphic profile of the model's kinetic curves changed dramatically between day 21 and day 27 post-challenge. The possible root causes for this profile change are addressed in Figure 2A.

Conclusions

In the POC imaging studies presented here, *Kinetics* consistently demonstrated an ability to detect and graphically present individual and group mean BL kinetic curve data for up to 10 BL mice simultaneously, in real time, at intervals of 1+ minutes, over an imaging time course of 30 minutes or more—and *Kinetics* achieved this through an easy-to-use, completely automated software protocol.

Consequently, *Kinetics* has the valuable potential of enabling investigators to routinely acquire and to report optimal, plateau-phase BL kinetic data from their BL animal models. It is hoped, therefore, that *Kinetics* will help promote an improvement in the quality of *in vivo* BL data presented in future, peer-reviewed journals.

References:

Inoue, Y., et. al., International J. of Biomed. Imaging, 2010, Article ID 471408.
Kim, M., et. al., Drug Metab. Dispos. 2022; 50: 277-286.
Sim, H., et. al. Cancer Res. 2011; 71(3): 686-692.