# Understanding Absolute Calibration

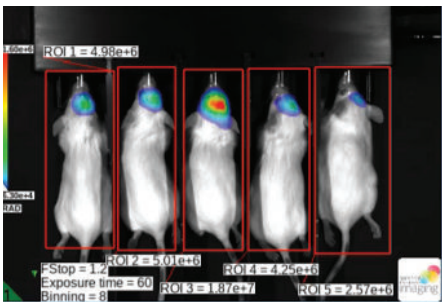
*Yes, you can (and should) change your acquisition settings … but only if you have absolute calibration!*

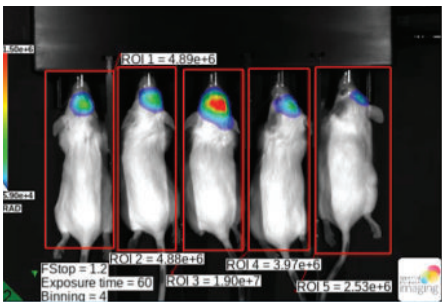
## What is Absolute Calibration?

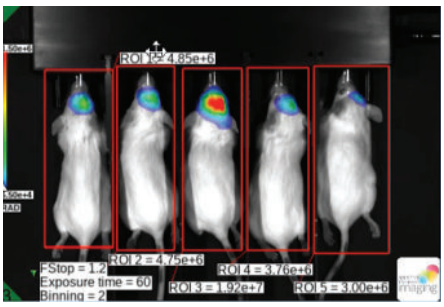
Absolute calibration is a process by which preclinical optical imaging systems (such as the Spectral Instruments Imaging Lago X, Ami HT or Kino) are calibrated so that the numerical pixel values directly correlate to a physical measurement of photons per second being emitted from that pixel area. This creates a standard unit of radiance that is consistent across different camera settings or even different instruments.

## Consistent Data Regardless of Acquisition Parameters

The data set below shows three images of five mouse brains taken using different camera settings, specifically using different binning levels: 8, 2, and 4. Despite the changing camera parameters, the ROIs (regions of interest) indicating brain signal are biologically identical, demonstrating that absolute calibration allows the photon measurement to stay consistent regardless of instrument acquisition settings or camera parameters.




## Practical Importance

### *Different Technicians Acquiring Data for the Same Study*

Absolute Calibration is practically important for users splitting imaging work between different technicians throughout the course of the study. In this scenario, one user may image using their preferred settings, and another user can image with different settings. As long as the instrument is absolutely calibrated, the changing acquisition parameters should not affect the quantitative photon measurement, so data remains comparable.

### *Ensuring Comparable Longitudinal Data with Optimal Sensitivity*

Over the course of an experiment, camera settings may need to be adapted week-to-week. In the example of a xenograft tumor model, early on the signal may be low, requiring high binning and long exposures, while later the intense signal may necessitate short exposures and low binning. Absolute calibration allows the freedom to modify settings as needed – to ensure optimal sensitivity and avoid saturation - without compromising data integrity. This also increases statistical power with smaller cohort sizes.

### *Comparing Data from Different Systems*

Whether you are comparing data from a system next door or from a collaborator on the other side of the world, in vivo imaging systems that are absolutely calibrated are designed to maintain data integrity cross platform.

## The Value of Absolute Calibration

Radiometric measurements made with *any* set of imaging parameters will return the absolute value

**Comparison of data over time**

Free to optimize camera settings troughout experiment without concern

Data taken years ago still valid

**Comparison of data across platforms**

Accurately compare data with collaborators around the world

Procedures and methods transfer

Easy transition from legacy imaging systems

**Ease of use**

The instrument tracks all parameters

Eliminates transcription, bookkeeping or calculation errors

## Summary

Absolute calibration links image pixel values to standardized physical radiance measurements. This creates quantitative, instrument-independent data that permits flexible modification of camera parameters without disrupting cross-system and cross-timepoint comparability. To that end, absolutely calibrated results vary only based on the biological signal intensity, not the device settings used during image acquisition. This enables quantitative comparison between measurements made at different times or even by different systems.