Detection Sensitivity

The parameters that control the number of photons collected (signal) and the image background (noise) determine the sensitivity of low light imaging. To maximize sensitivity, the goal is to increase signal and decrease background

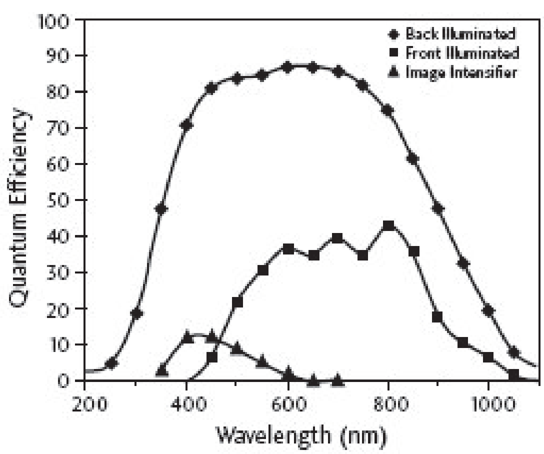
Several factors affect the number of photons collected, including the lens f/stop, image magnification, size and detection efficiency (quantum efficiency) of the CCD, transport efficiency of the imaging optics, and the image exposure time.

CCD Detection Efficiency

IVIS® Imaging Systems use a back-thinned, back-illuminated CCD cooled to -90 °C to -105 °C (depending on the system). This type of CCD provides high quantum efficiency of over 80% across the visible and near infrared part of the spectrum.

Figure 1 shows detection efficiencies for several commonly used photon detectors. The back-illuminated CCD has the highest efficiency, particularly in the 600-800 nm region of the spectrum, the area of greatest interest for *in vivo* imaging.

Figure 1 Quantum efficiencies



Bialkali photocathode (▲)
Back-illuminated CCD (◆)
Front-illuminated CCD (■).

IVIS systems use back-illuminated CCDs.

Lens Aperture

IVIS Imaging Systems are equipped with a high-light-collection f/1 lens. The sensitivity of the IVIS Imaging System can be adjusted by changing the f/stop setting that controls the lens aperture. The detected signal scales approximately as $1/(f/stop)^2$. For maximum sensitivity, select f/1, the largest aperture setting on the IVIS Imaging System (Figure 2). This provides the greatest light collection efficiency, but results in the minimum depth of field for the image. The depth of field refers to the depth over which the image appears to be in focus and is determined by the f/stop and the field of view (FOV).

At f/1, the depth of field ranges from ~0.2 cm at FOV= 3.9 cm (IVIS Imaging System 200 Series only) to ~2 cm at FOV= 25 cm. You can use the manual focus option on the Control panel to easily assess the depth of field at any f/stop and FOV setting. Generally f/1 is recommended for low light luminescent images and f/2 or f/4 is recommended for brighter luminescent or fluorescent images.

Figure 2 Lens f/stop positions.

Left: lens wide open at f/1; right: lens closed down at f/8.

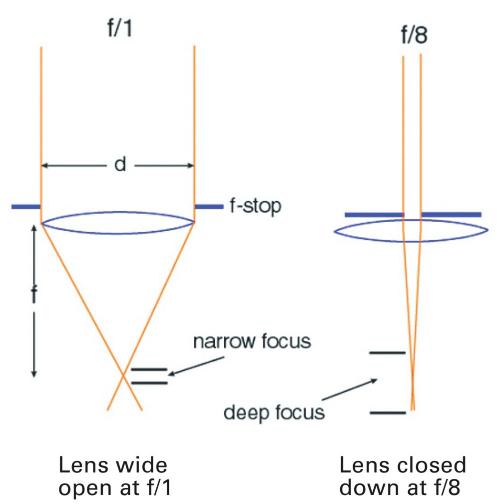


Image Exposure Time

The image exposure time also affects sensitivity. The number of photons collected is directly proportional to the image exposure time. For example, an image acquired over a two minute exposure contains twice as many detected photons as an image acquired over a one minute exposure.

Longer exposure times are usually beneficial when imaging very dim samples. However, this may not always be true because some types of background, dark charge in particular, increase with exposure time. For more information on backgrounds, see Concept Tech Note *3, Luminescent Background Sources and Corrections*.

An IVIS® Imaging System has extremely low background that enables exposures of up to 30 minutes. However, animal anesthesia issues and luciferin kinetics limit practical exposure times for *in vivo* imaging to 5-10 minutes.

Field of View (FOV)

The FOV indirectly affects sensitivity. Changing the FOV without changing the binning or the f/stop does not significantly affect sensitivity. However, CCD pixels are effectively smaller at a smaller FOV (higher magnification) so that higher levels of binning can be applied without loss of spatial resolution.

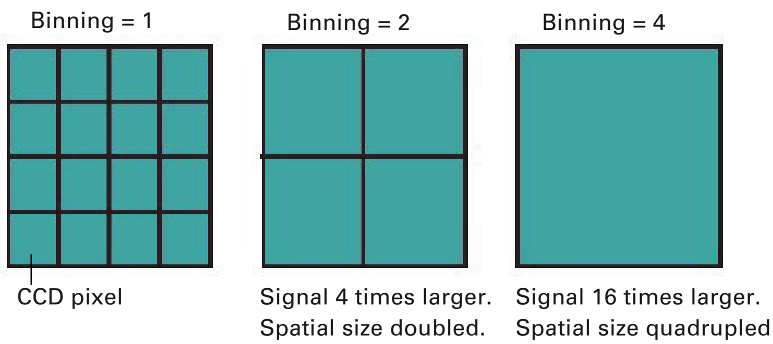
For example, an image acquired at binning=4 and FOV=20 cm has the same spatial resolution as an image acquired at binning=8 and FOV=10 cm. Due to the increase in binning, the latter image has a four-fold increase in sensitivity compared to the former.

Binning

A charge coupled device (CCD) is a photosensitive detector constructed in a two-dimensional array of pixels. After an image is acquired, each pixel contains an electrical charge that is proportional to the amount of light that the pixel was exposed to. The software measures the electrical charge of each CCD pixel and assigns a numerical value (*counts*). For more information on counts and other measurement units, see the Concept Tech Note 2, *Image Display and Measurement*. The resulting image data comprise a two-dimensional array of numbers; each pixel contains the counts associated with the amount of light detected.

IVIS® Imaging Systems are equipped with a CCD that ranges from 1024×1024 to 2048×2048 pixels in size, and thus have a high degree of spatial resolution. At binning=1, each pixel is read and the image size (number of pixels) is equal to the physical number of CCD pixels (Figure 3).

Figure 3 A small segment of the CCD.
At binning = 2, 4 pixels are summed together; at binning = 4, 16 pixels are summed.



At binning=2, four pixels that comprise a 2×2 group on the CCD are summed prior to read out and the total number of counts for the group is recorded (Figure 3). This produces a smaller image that contains one fourth the pixels compared to an image at binning=1. However, due to summing, the average signal in each pixel is four times higher than at binning=1. At binning=4, 16 pixels are summed prior to read out.

Binning significantly affects the sensitivity of the IVIS Imaging System. Binning at higher levels (for example, ≥ 4) improves the signal-to-noise ratio for read noise, an electronic noise introduced into the pixel measurement at readout. If four pixels are summed before readout, the average signal in the summed pixel (*super pixel*) is four times higher than at binning=1.

The read noise for the super pixel is about the same as it was for the individual pixels. Therefore, the signal-to-noise ratio for the read noise component of the image noise is improved by a factor of four. Read noise is often the dominant source of noise in *in vivo* images, so a high binning level is a very effective way to improve the signal-to-noise ratio.

Unfortunately, binning reduces the spatial resolution in an image. For example, at binning=2, a super pixel is twice as wide as a pixel at binning=1. This results in a factor of two loss in image spatial resolution. However, for *in vivo* imaging, the added sensitivity is usually more important than the spatial resolution. Further, since *in vivo* signals are often diffuse due to scattering in tissue, little is gained by increasing spatial resolution. For more background on the propagation of light through tissue, see Concept Tech Note 7, *Planar Spectral Imaging*. In such cases, high levels of binning may be appropriate (up to 10 or 16, depending on the CCD of the IVIS Imaging System). If signal levels are high enough that sensitivity is not an issue, then it is better to image at a lower binning level (two or four) in order to maintain a higher degree of spatial resolution.

NOTE: For application-specific questions regarding the appropriate binning level, please contact Caliper technical support.

The IVIS Acquisition Control panel provides several binning options. The actual binning numbers associated with these settings depends on the CCD chip and type of image (Table 1). These choices should satisfy most user needs. However, if you want to manually control binning, you can specify **Manual Binning** in the Living Image Tools-Preference-Camera Settings box.

Table 1 Binning settings

Binning	**IVIS® System**			
	100/200/Spectrum[1]	**Lumina**	**Lumina XR**	**Lumina Kinetic**
Small (high-resolution) Lumin	Bin 4	Bin 2	Bin 2	Bin 2
Medium Lumin[2]	Bin 8	Bin 4	Bin 4	Bin 4
Large (high-sensitivity) Lumin	Bin 16	Bin 8	Bin 8	Bin 8
Small (high-resolution) Photo	Bin 2	Bin 1	Bin 1	Bin 1
Medium Photo[2]	Bin 4	Bin 2	Bin 2	Bin 2

[1] Some early IVIS 100 Systems with Spectral Instruments SITe cameras, and all Roper and Princeton Instrument cameras, are not supported in Windows 7/Living Image 4.2 software.

[2] Default setting

You can also apply *soft binning* after an image is acquired. Conceptually, soft binning is the same as hardware binning— groups of pixels are summed and a smaller, lower resolution image is produced. However, in soft binning the summing is performed digitally on the stored image data, not on the electronic charge before readout as in hardware binning.

Although soft binning does not improve the signal-to-noise ratio for read noise, it may enhance the signal visibility because it reduces the statistical scatter of nearby pixel contents. Usually, hardware binning is preferred, but if it is not possible to take another image, applying soft binning to the data may provide a worthwhile solution.

Smoothing

Smoothing is a filtering method that reduces noise in the image data. To apply smoothing, the software replaces the intensity of each pixel with the average intensity of a nearby pixel neighborhood that includes the pixel. Figure 4 shows a 3x3 pixel neighborhood.

Smoothing does not change the pixel size and helps:

- Eliminate outlier pixel values that are extremely high or low.
- Reduce noise (fluctuations) in the image to help reveal small signals.

Figure 4 3x3 pixel neighborhood