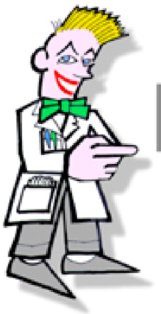

Handy Helper

Application Note 04

Digitisation and Data Dynamic Range
What is the Difference?

This application note has been written to provide a guide to the terminology used to define “dynamic range”, and the important technical factors which CCD imaging systems require to generate accurate and linear scientific data.

Gel documentation and analysis systems available today use a wide range of CCD cameras for image acquisition. The increasing use of chemiluminescence has also led to an advance in the type of cameras now being utilised, especially in those applications that demand a high level of quantitative accuracy.

Data Dynamic Range

True data dynamic range is defined as the maximum range of emission intensities varying between faint bands/spots and bright bands/spots that, when detected and quantified by a CCD imaging system, provide a linear data relationship. This is commonly referred to as a linear OD (Optical Density) range.

Traditionally, film has always been used to develop chemiluminescent blots. This has a limited linear dynamic range of only 0 - 1.8 OD. Alternatively, the G:BOX ChemiHR16 offers a very wide data dynamic range of 0 - 4.8 OD. Several technical factors contribute to data dynamic range including:

- **Noise Readout Rate of the CCD Camera** - This is commonly referred to as electrons / second and is a function of the noise characteristics of the CCD sensor, cooling temperature, and camera electronics. CCD sensors in Syngene image capture systems are rated very low compared to alternative systems that display up to 500X higher noise levels.

- **CCD Cooled Temperature and Dark Current Noise** - Deep cooling lowers the dark current noise level of the CCD sensor to increase data dynamic range. CCD sensors in Syngene image capture systems are cooled by a minimum of 25C compared to some alternative technologies that only cool by 10C.

- **Pixel Well Capacity** - Commonly referred to as the number of electrons that a pixel can ‘hold’ until saturation occurs. The G:BOX ChemiHR16 can also utilise a pixel binning process that increases this whilst maintaining extremely low noise levels. Although alternative CCD sensors can provide a wider well capacity that extends up to 200,000 electrons, the true data dynamic range is reduced due to high noise characteristics of the CCD, resulting in significant electron noise.

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Digitisation

CCD sensors (Charge Coupled Device) detect light (photons) striking the individual pixels of the sensor. These photons are then converted to electrons, which accumulate in the wells of the pixels generating an analogue or video signal. This analogue signal is then digitised either in the electronic head of the camera or in a digitising card within a computer. This process is commonly referred to as an analogue to digital conversion (A/D conversion). Cameras have different types of converter that can be described as 8, 10, 12 or 16 bit. Quite simply the number of bits is an indication of the number of measuring graduations that are used in the conversion.

8 bit gives 256 shades of grey = 2^8
10 bit gives 1,024 shades of grey =2^{10}
12 bit gives 4,096 shades of grey =2^{12}
16 bit gives 65,536 shades of grey =2^{16}

Thus, a given data dynamic range can be digitised to several digitised bit depths. In general, the higher the bit depth, the higher the equipment costs and the slower the CCD readout rate becomes, making focusing and sample positioning difficult. However, the G:BOX ChemiHR16 system will provide a Real Time Readout despite the 16 bit depth, in comparison to 1 frame every 3-7 seconds in some competitive 12 and 16 bit technologies.

In reality, the images displayed on the computer screen (and thermal printer) will of course only be seen as 256 shades of grey. Therefore there will be no visible difference between the images seen.

However, the analysis software can distinguish between the levels of grey and of course the higher the number of levels then the greater the accuracy, especially if working in quantitative modes.