Not All Bits Are Created Equal

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Manipulating Bits — A Gray Area

Scientific data collected with an imaging device can be true or altered. Alteration of imaged data is used to create an impression of more data retrieved than is actually collected. Altered data can be accomplished either through software or through digitization. Both methods are able to compress or spread out true data, but the bottom line is that the true data is not accurately represented after alteration.

In a charge-coupled device (CCD) camera, bits represent the range of quantitative data obtained from an image. The data is recorded in distinct shades of gray ranging from absolute white to absolute black. The number of shades of gray is denoted by the bit number of a camera; an 8-bit camera has 2^8, or 256, distinct shades of gray, a 12-bit camera has 4,096 (2^{12}) shades of gray, and a 16-bit camera has 65,536 (2^{16}) shades of gray. Thus, a 16-bit camera is said to have a greater dynamic range than the 12- or 8-bit camera. For reference, the human eye is capable of distinguishing 64 levels of gray (that is, 2^6), whereas the computer monitor screen displays 256 levels (2^8); in other words, any image displayed by a computer will be 8-bit, but even though more data is available, we can only distinguish a quarter of it (Figure 1).

A higher-bit system is able to distinguish minute differences in imaged data better than a lower-bit system. A 16-bit digital image is much "deeper" than a 12-bit and is therefore more beneficial for quantitative analysis. This ability corresponds to the ratio of the number of gray levels between the two systems. In a 12-bit camera, a change of 1 level of gray would correspond to a change of 16 ($2^{16}/2^{12} = 2^4$) levels of gray in a 16-bit camera. Obviously a higher-bit system is more desirable, since the data output is much more sensitive to subtle differences within an image.

Alteration By Software with File Generation

The first and simplest way in which imaged data can be boosted is via software, which takes lower-bit data and converts it to a higher-bit file, and is known as "x"-bit file generation. Creating a higher-bit file distorts the actual data obtained. For example, in an image acquired with a 12-bit camera, one of the pixels might have a value of 2,000 on the gray-level scale with its range of 0 to 4,095. Converting that pixel into a 16-bit file would convert its value to 32,000 in a range of 0 to 65,535. This newly created image is read out as an impressive 16-bit file, but the data is falsely represented — the file cannot contain any more information than the capacity of the 12-bit camera (Figure 2).

CCD Collection and Transfer

Another manner in which larger-bit images are generated is dependent on the way a CCD camera collects and transfers data. A CCD chip measures light intensity by generating and collecting electrons. The best analogy for a CCD chip is an array of electron-filled buckets called pixels (Figure 3). Each bucket has a certain depth; some are shallow and hold 15,000 electrons, others are able to contain 40,000 electrons, and some, up to 300,000 electrons. This capacity is the well depth of the chip.

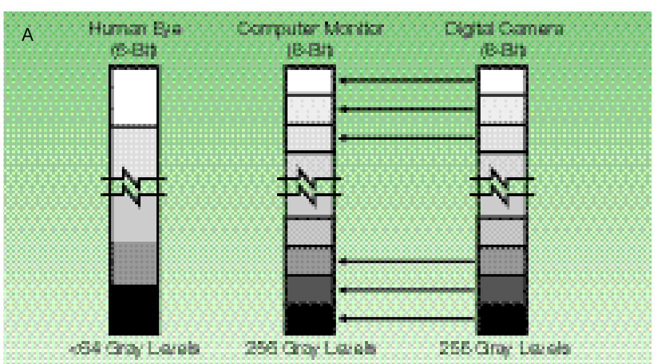

Fig. 1. The 256 shades of gray that constitute an 8-bit image are mapped directly to the computer monitor, which in turn is viewed by the human eye.

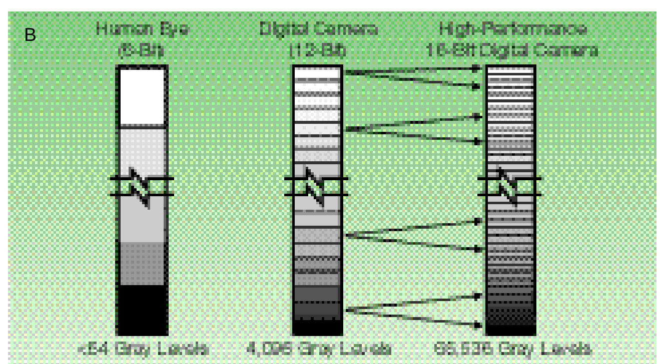

Fig. 2. Converting data from 12-bit to 16-bit distorts the true data acquired.

CCD chip designs govern the capacity of their buckets. The two designs most commonly seen are the full-frame sensor and the interline chip. The first, the full-frame sensor (Figure 3), is a straightforward array of pixels of various capacities (well depth), and widths (pixel size). The other design, the interline chip, introduces a feature that is beneficial in the transfer rate but limits the pixel size. The interline chip, although also laid out as an array of pixels, includes transfer sections located next to each row of pixels. Due to its layout, the parameters of each pixel are restricted. It is easy to see the limitations of the bucket size in the architecture of the interline chip. Construction of a megapixel CCD chip with pixels adequately large for high-quality data acquisition is technically possible; however, the chip would be very large and therefore very expensive. As a result, such a chip is not being offered commercially.

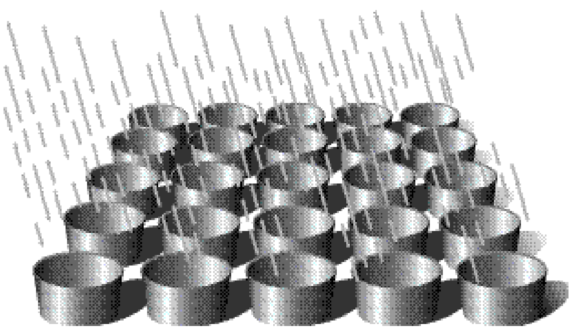

Fig. 3. The pixels of a CCD collect light and convert it into electrons.

Once charge has accumulated within the pixels, it is transferred out of the pixels for conversion into data. An analog-to-digital converter, or digitizer, translates the number of electrons into digital numerical data. The digitizer determines the bit number of the output. That is, a 12-bit digitizer will produce a 12-bit image and a 16-bit digitizer a 16-bit image. To ensure accurate data collection, it is crucial to select a digitizer that appropriately matches the well depth of the pixels. For a 12-bit digitizer with 4,096 levels, the best well depth would have a capacity of 4,096 electrons; that is, for each collected electron, the data would correspond literally to a gray level. For example, if a pixel contained 2,000 electrons, the digital readout would be at the 2,000th gray level.

Noise

A factor that needs to be considered for accurate digitization is noise. Noise is added signal from sources such as dark current, shot noise, and read noise, that is not part of the signal from the object being imaged. Noise reduces the dynamic range, which is the ratio of well depth to noise:

$$\frac{\text{well depth}}{\text{noise}} = \text{dynamic range}$$

For example, a system with a well depth of 40,000 electrons and with a noise of 10 electrons would only have a dynamic range of 4,000 electrons. (Other examples are given in the table.)

With all this in mind, the true dynamic range and the system's digitizer together determine the true bit output. Following the above example, the best output would be as follows: For a CCD chip with a calculated dynamic range of 4,000 electrons, a 12-bit digitizer would allocate that to 4,096 gray levels. If the same information were to be translated by an 8-bit digitizer, the 4,000 electrons would need to be compressed into 256 gray levels, causing loss of information. Conversely, creating seemingly impressive data by the use of a 16-bit digitizer distorts the true data obtained into 65,536 gray levels, while adding no additional information.

Bio-Rad's Approach

Bio-Rad's imaging instruments provide customers the truest data possible. We understand that true data is the most important part of your research. All of our CCD camera systems output data without computer-aided enhancements or digitization mismatching, ensuring accurate data acquisition. We acquire the best data by using large well depths and reducing noise. By clearly stating an ambient or true cooling method, and with back thinning (where appropriate), we excel at lowering noise. To maximize the usefulness of your imaging systems, be aware of where your bits are coming from.

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Well Depth Comparison

Pixel Size (µm)	Well Depth (Pixel Charge Capacity)	Typical Camera Noise	CCD Dynamic Range	Appropriate Digitizer
6.8 x 6.8	45,000 electrons	10 electrons	4,500:1	12-bit (4,096:1)
9 x 9	85,000 electrons	16 electrons	6,071:1	12-bit (4,096:1)
24 x 24	330,000 electrons	6 electrons	55,000:1	16-bit (65,000:1)