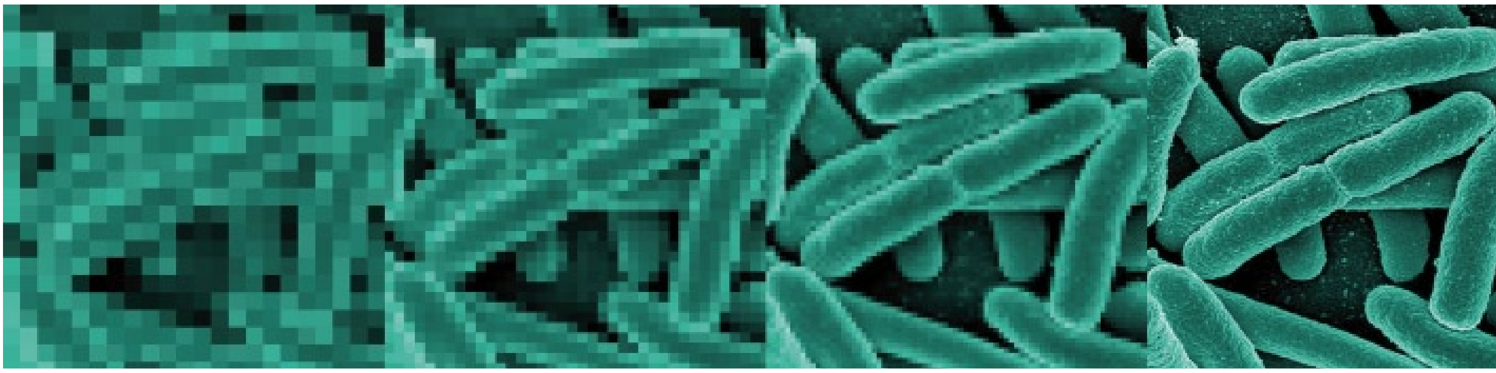
Fundamental Knowledge

Part 4
Digitial Imaging Cameras: Deciphering Specifications



In the first instalments of this article series we looked at the theory behind microscopy: how a small facet of a sample can be magnified and focused efficiently and effectively at the focal plan of the user's eyes or the imaging device. In this article, we look at the surprisingly complex world of digital microscopy cameras and how to find the best one for each research task.

Digital microscope cameras are designed with microscopy in mind and, as such, are specified quite differently to an average consumer camera. Furthermore, microscope cameras are designed to be in-use and 'online' for long periods of time and are therefore more robust. There are a number of features that have a great bearing on which microscope camera to choose, such as pixel size, binning modes, data transfer technology and dynamic range for example. In this article we look at demystifying some of these terms so that users can select the right camera for their research.

Time Related Features

As high-performance camera systems, typically employing low-noise cooled charge-coupled device (CCD) detectors, have become more capable of capturing even relatively weak signals at standard video rates and higher, certain performance factors necessarily take on greater importance. A camera system's readout rate and frame rate are interrelated parameters that are crucial to the ability of the system to record specimen data at high temporal frequency.

Read-out-speed

Read-out-speed is governed by the time required to digitise a single pixel (the serial conversion time) and is defined as the inverse of that value, i.e. how many digitisations are completed (or pixels read-out) per second. The rate is often stated as a frequency (hertz, Hz), and some camera manufacturers refer to this specification as 'pixel clock rate' or simply 'clock rate'.

Frame-rate

The frame rate of an imaging system incorporates the exposure time and extends the single pixel readout rate to the entire pixel array. It is defined as the inverse of the time required to acquire an image and to completely read the image data out to the amplifier and subsequent transfer to the PC. This variable is typically stated in frames per second (fps) or in frequency units (Hz). A number of basic operations typically contribute to the frame acquisition and frame read time intervals, and these are listed and discussed further below

Exposure Time

This is the amount of time used to capture each frame, such that fast events or bright samples will only need short exposure times where as samples with low light levels need longer exposure times to capture enough light information to provide an image.

Table 1: Bit depth and dynamic range (approximate) of charge-coupled devices

Bit Depth	Greyscale Levels	Dynamic Range (Decibels)
1	2	6 dB
2	4	12 dB
3	8	18 dB
4	16	24 dB
5	32	30 dB
6	64	36 dB
7	128	42 dB
8	256	48 dB
9	512	54 dB
10	1,024	60 dB
11	2,048	66 dB
12	4,096	72 dB
13	8,192	78 dB
14	16,384	84 dB
16	65,536	96 dB
18	262,144	108 dB
20	1,048,576	120 dB

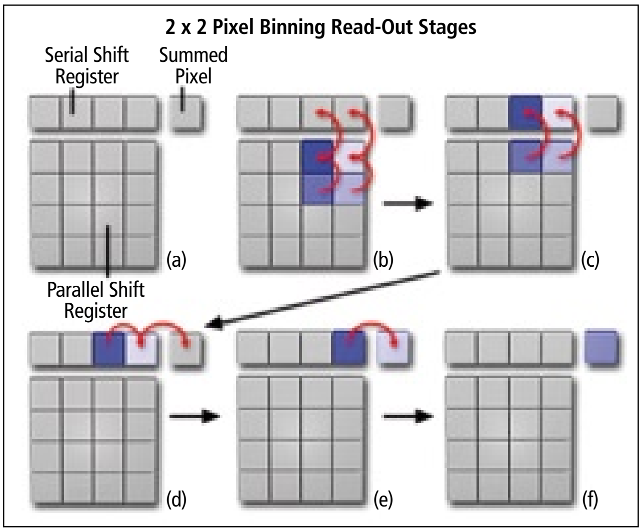


**Fig. 1: A outline of 2 x 2 pixel binning. A schematic drawing of a 4 x 4 parallel shift register pixel array is illustrated in Figure 1(a), along with a four-gate serial shift register and summing pixel or well (also termed an output node). Illuminating photons impact the CCD photodiodes, creating a pool of electrons that accumulates in each pixel, shown in Figure 1(b) as a cluster of four blue-shaded squares in the upper right hand corner of the parallel shift register. The number of electrons that each pixel can accommodate is termed the well depth and ranges from about 10,000 to 350,000, depending upon the CCD specifications. Binning can be used to increase focusing accuracy by reducing the time necessary for image acquisition, while providing greater sensitivity to lower out-of-focus light levels. To illustrate this process, Figure 1(b) shows each integrated pixel in the parallel register stepping by an increment of one gate to yield the arrangement shown in Figure 1(c). Here, the electrons from two pixels remain in the parallel shift register, while those from the other two have been transferred to the serial shift register. Another step (Figure 1(c)), shifts the remaining electrons in the parallel shift register to fill the adjacent gate elements in the serial register (Figure 1(d)). The final steps involve shifting of charge from the serial register, two pixels at a time, to the summing pixel (Figure 1(d) and (e)). Figure 1(f) illustrates the combined charge of four pixels in the summing well awaiting transfer to the output amplifier, where the signal will be converted to a voltage and then transferred to other integrated circuits for further amplification and digitisation. The process continues until the entire array has been read out.
In this example, the area of four adjacent pixels has been combined into one larger pixel, sometimes referred to as a super pixel. The signal-to-noise ratio has been increased by a factor of four, but the image resolution is cut by 50%.**

Resolution Related Features

Number of Pixels

The number of pixels is usually expressed as a horizontal x vertical ratio such as 2560 x 1920 for the Olympus DP25. The result of this ratio gives the overall number of pixels in the chip, which for this example is 4,915,200 pixels or 4.9 megapixels. The effective number of pixels can be different though; for example the Olympus DP71 uses a 'Pixel-Shift' system with a 1360 x 1024 chip to produce 4080 x 3072 effective pixels (12.5 megapixels)

Pixel Binning

Pixel binning is a clocking scheme used to combine the charge collected by several adjacent CCD pixels, and is designed to reduce noise and improve the signal-to-noise ratio and frame rate of digital cameras. The binning process is performed by on-chip timing circuitry that assumes control of the serial and parallel shift registers prior to amplification of the CCD analog signal. For example, a 2 x 2 binning means that the outputs from a matrix of two horizontal and two vertical pixels (4 pixels in all) is outputed as if it were one pixel. As a result, both the vertical and horizontal resolutions are halved – for example a 1360 x 1024 chip in 2 x 2 binning mode will have an effective output of 680 x 512 pixels. Binning is used to increase sensitivity in low light levels for example, as well increasing frame rate for either live mode or documentation, since it effectively combines the output from a set number of pixels and reads it out as if they were one super pixel. This does though reduce the resolution.

Sensitivity Related Features

Pixel Size

Pixel size is reported as a measure of the width and height of each pixel. On the Olympus XM10 for example each pixel has a 6.45 x 6.45 µm, whereas the DP25 has a pixel size of 3.4 x 3.4 µm. Both cameras use the same size chip (2/3 inch) but due to the smaller pixel size, the DP25 has many more pixels. This does improve resolution, but its main effect is on sensitivity. The smaller the individual pixel, the less sensitive it is to incoming light. Therefore sensitivity decreases in proportion to pixel size, whereas resolution increases.

Bit Depth

Bit depth refers to the binary range of possible greyscale values used by the analogue to digital converter, to translate analogue image information into discrete digital values capable of being read and analysed by a computer. Considering then that most cameras are colour cameras, it provides a measure of the number of shades of a specific colour that each pixel in a chip can differentiate. The actual number of shades is given by 2^X where 'X' is the number of bits, therefore a 10 bit pixel can discriminate between 1024 shades. The Olympus XC10 colour camera provides a bit depth of 3 x 12 bits, so that is 12 bits per RGB channel (Red, Green and Blue). Therefore each pixel can discriminate 4096 shades of its designated colour. The bit depth can sometimes be given as a composite number, where the number of bits for each colour channel is combined, such that the XC10 might be listed as 36 bit.

Dynamic Range

Dynamic range is the ratio of the maximum light intensity measurable (at pixel saturation), to minimum light intensity measurable (above read-out noise). Often reported in decibels, this represents the range of values between the minimum and maximum number of photons that a pixel could discern. Factors such as read-out noise, saturation and bleed-through are important here. It is also worth noting that pixel size will influence dynamic range, such that smaller pixels saturate more quickly than larger pixels. Furthermore, temperature and integration/exposure time affect dynamic range as shown in figure 3.

Table 1 presents the relationship between the number of bits used to store digital information, the numerical equivalent in greyscale levels, and the corresponding value in decibels (one bit equals approximately 6 dB). As illustrated in the table, E. g. if a 0.72 volt video signal were digitised by an A/D converter with 1-bit accuracy, the signal would be represented by two values, binary 0 or 1 with voltage values of 0 and 0.72 volts. If the digitiser employs an 8 bit A/D converter, (256 discrete greyscale levels), to represent the voltage amplitudes, a maximum signal of 0.72 volts would then be subdivided into 256 steps, each step having a value of 2.9 millivolts.

Colour and Size Related Features

Sensor Type

Most people are aware of the existence of charge-coupled device (CCD) and complementary metal oxide semiconductor (CMOS) imaging sensors, although most

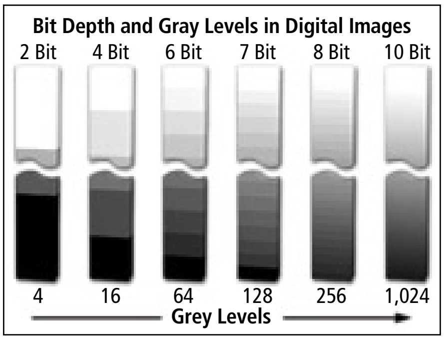


Fig. 2: Schematic representation of the visual effect of different bit depths

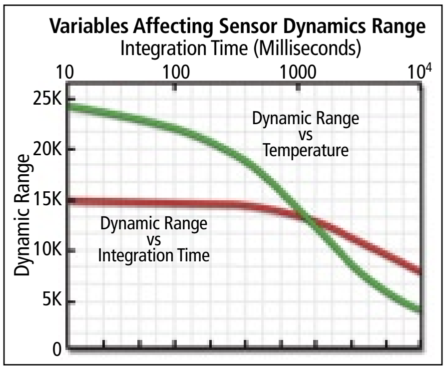


Fig. 3: Graph showing the effect of integration time and temperature on the dynamic range of a typical pmaging chip

don't know the difference. The main difference lies in where the conversion of the signal occurs. Whatever the technology in use, image capture requires the conversion of: photons to electrons (p → e), then electrons to voltage (e → V) and then voltage (analogue) to bits (digital) (a → d). CCD chips perform p → e at the pixel level, then perform e → V on the imaging chip (not at the pixel) and a → d on a separate circuit board. CMOS sensors perform both p → e and e → V at the pixel level and a → d on the imaging chip and so do not require a separate circuit board. Some CCD cameras used in scientific applications are operated at room temperature while others are cooled to reduce dark current (a 20 °C decrease in temperature reduces the dark current of the CCD ten-fold). Because the charge storage wells do not fill with thermally-generated dark noise during the integration period, longer exposures are possible. Cooled cameras for scientific use are often designated slow-scan because their frame rate is less than that of a standard video camera. Where originally CCDs outperformed CMOS chips in terms of image quality, nowadays there is no clear line dividing them.

CCD Chip Designs

Two CCD designs are commonly used to achieve rapid transfers: the interline-transfer CCD and the frame-transfer CCD. The interline-transfer CCD incorporates charge transfer channels (termed 'interline masks') immediately adjacent to each photodiode so that the accumulated charge can be efficiently and rapidly shifted into the channels after image acquisition has been completed. Interline-transfer CCDs can be electronically shuttered by altering the voltages at the photodiode so that the generated charges are injected into the substrate rather than shifted to the transfer channels. These devices also include an electron 'drain' to prevent blooming and are usually equipped with microlens arrays to increase the photodiode fill factor and quantum efficiency.

The frame-transfer CCD uses a two-part sensor in which one-half of the parallel array is used as a storage region and is protected from light by a light-tight mask. Incoming photons are allowed to fall on the uncovered portion of the array and the accumulated charge is then rapidly shifted into the masked storage region for charge transfer to the serial output register. While the signal is being integrated on the light-sensitive portion of the sensor, the stored charge is read out. A disadvantage of this architecture is charge smearing during the transfer from the light-sensitive to the masked regions of the CCD, but this can often be compensated for.

Becoming more important with interline CCDs, is the 'mode' or how the camera reads the image off the chip i. e. interlaced or progressive. Taking a 1376 x 1024 imaging chip for example: there are 1024 horizontal lines of pixels. In interlaced mode, lines 1, 3, 5, 7, …, 1023 are read first and then lines 2, 4, 6, 8, …, 1024 are read second and consequentially the full frame is generated with every 2 read-outs. In progressive mode, every line of pixels is read-out at the same time so the full frame is available with each read-out.

Monochrome or Colour

Digitial imaging camera chips are colour blind. Each pixel is a specialised element to convert photons of light into electrons which are then read off of the chip based on the principles and settings mentioned above. It is easy to see from this then that monochrome cameras are reasonably straight forward but how is it possible for a camera chip to discern different colours? This can be achieved in two different ways:

- Single chip – places a Bayer (or mosaic) filter over the pixels such that each pixel will receive red or blue or green photons only. This is the most cost efficient way of achieving colour images with a digital camera. Due to the way the pixels are aligned a single chip colour camera will have ¼ of its pixels devoted to red, a ¼ to blue and ½ to green. This means therefore, that a certain amount of interpolation is performed in order to recreate an image in the computer, since each pixel can only report a colour value for its corresponding colour, whereas the actual sample provides a colour value for each colour at each pixel. Interpolation equations are very good and can still provide high quality images.
- Multi-chip – uses a prism to separate and direct the light to three separate imaging chips, one for each RGB colour channel. This means that each pixel position can provide a direct readout for each colour without any interpolation. These multi-chip cameras therefore provide much better image clarity due the collection of a greater amount of data.

Conclusions

Selecting the right digital imaging camera for your application(s) is not necessarily as straight as it may at first seem. There are many important features to be considered, many of which are briefly discussed here. More in depth information for each of these is available from the excellent Florida State University 'Molecular Expressions' Microscopy Primer – http://micro.magnet.fsu.edu/primer/.

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