

# A scientific approach to Imaging

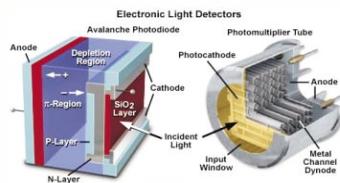
CCD cameras are used not only in surveillance systems, but also in various scientific applications. **CCTV focus** gives such applications equal importance as in security. The following article is an extract of a larger text, written by **Kenneth R. Spring** and **Michael W. Davidson**, and is reproduced courtesy of the Florida State University. For all readers that will find this article interesting and informative, we recommend visiting their web site at [microscopy.fsu.edu](http://microscopy.fsu.edu), which also includes some brilliant Java scripted animations that give their text an extra dimension

Over the past several years, the rapidly growing field of fluorescence microscopy has evolved from a dependence on traditional photomicrography using emulsion-based film to one in which electronic images are the output of choice. The imaging device is one of the most critical components in fluorescence microscopy because it determines at what level specimen fluorescence may be detected, the relevant structures resolved, and/or the dynamics of a process visualized and recorded.

The range of light detection methods and the wide variety of imaging devices currently available to the microscopist make the selection process difficult and often confusing. This discussion is intended to aid in understanding the basics of light detection and to provide a guide for selecting a suitable detector for specific applications.

## Imaging Detector Characterization Parameters

Electronic imaging sensor performance



may be described by a number of variables including: **spectral sensitivity, quantum efficiency, spatial resolution, uniformity, the signal/noise ratio, dynamic range, and response speed.**

**Spectral sensitivity** refers to the detector signal as a function of the wavelength of the incident light. This parameter is often expressed in terms of the **quantum efficiency** (QE), a measure of the detector's ability to produce an electronic charge from the percentage of incident photons that are detected. The **limiting spatial resolution** is commonly determined from the minimum separation required for discrimination between two high contrast objects, for instance, white points or lines on a black background. Contrast is an important factor in resolution because high contrast objects (e.g. black and white lines) are more readily resolved than low contrast objects (e.g. adjacent gray lines).

More informative measures of the spatial resolution of an electronic detector are the **modulation transfer function** (MTF) and

the **contrast transfer function (CTF)**, both of which demonstrate the magnitude of the detector response as a function of spatial frequency. **The CTF is determined from the detector response to a series of black and white bars that become progressively narrower and closer together.** Each pair of bars is essentially a square wave with 100 percent contrast. **The MTF is an expression describing the reduction in contrast of a sinusoidal signal (60% contrast sine waves) as a function of spatial frequency.** The limiting resolution of an electronic detector is the smallest target size that is detectable above the noise threshold, a concept that is often referred to as the frequency of limiting resolution, which is the spatial frequency for which the MTF falls to a value of 3 percent, corresponding to the limit of visible detection.

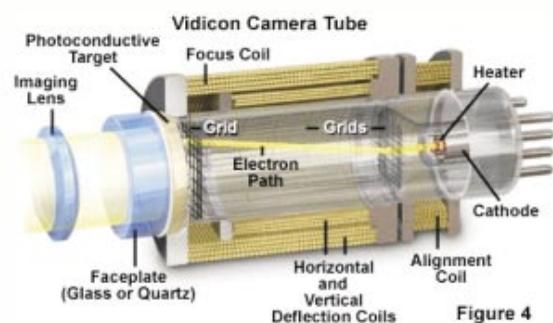
The **uniformity** of electronic detectors encompasses several variables: **gain variations** across the sensor, **regional differences** both in noise and sampling efficiency (often termed **shading**), and **spatial variation** in the efficiency of light collection or transmission. Electronic detectors are often compared by their **signal/noise** ratio (designated S/N), a measure of the variation of a signal that indicates the confidence with which the magnitude of the signal can be estimated.

Visible light has an **inherent noise** component arising from the stochastic nature of the photon flux, which is equal to the square root of the signal. Noise also derives from a variety of other sources such as the output amplifier (read noise), and in electronic devices can often be reduced by lowering the operating temperature. Noise arising in electronic devices in the absence of light is termed dark current or dark noise, which is thermally sensitive, increasing as a function of the detector temperature.

Intrascene **dynamic range** is derived from the maximum and minimum intensities that can be simultaneously detected in the same field of view. This quantity represents the range of intensities that can be accommodated when detector gain, integration time, lens aperture, or other variables are adjusted for differing fields of view. The terms dynamic range and signal/noise should not be confused. Dynamic range is often calculated as the maximum signal that can be accumulated divided by the noise associated with reading that signal. The response speed of an electronic detector is described by its lag, representing the fraction of the previous image that carries over into the next one after a prescribed time interval has elapsed.

### Area detectors

These devices are generally divided into two categories: **tube-type** and **solid state** detectors. The vidicon tube camera (illustrated in Figure 4) is a tube-type detector in which the photosensitive surface is “read out” by a scanning electron beam. In vidicons, the photosensitive surface stores charge rather than liberating electrons as in a photocathode. Photons captured by the photosensor alter its electrical resistance at their site of impact, and the current of the scanning beam flows more readily through



these sites generating a signal. Because vidicon tube sensors have been largely supplanted by modern solid-state detectors and are of interest only due to historical significance, they will not be considered in any

further detail.

**Solid-state detectors** consist of a dense matrix of photodiodes incorporating charge storage regions. Several variations on the basic concept are commercially available including the popular **charge-coupled device** (CCD), the **charge-injection device** (CID), and the **complimentary-metal-oxide-semiconductor** detector (CMOS).

In each of these detectors, a silicon diode photosensor (often denoted a **pixel**) is coupled to a charge storage region that is, in turn, connected to an amplifier that reads out the quantity of accumulated charge. In the CID and CMOS detectors, **each individual photosensor has an amplifier** associated with it and the combined signal from a row of amplifiers is output in parallel. Although techniques for charge storage by silicon photodetectors were known for many years before the development of the CCD, a suitable mechanism for the systematic read out of this stored charge needed to be devised before the device became a reality. **In a CCD, there is typically only one amplifier at the corner of the entire array**, and the stored charge is sequentially transferred through the parallel registers to a linear serial register and then to an output node adjacent to the read-out amplifier.

Because the CCD is presently the **most widely used detector**, we will consider its performance in more detail. Distinctions will be made, where appropriate, between the two classes of CCD cameras: consumer-grade and scientific-grade. It is important to point out that although all electronic detectors are **analog devices** that generate electrical currents or charges, cameras with an internal digitizer have recently been denoted digital cameras because they do not have an analog signal output.

Some CCD cameras used in scientific applications are operated at **room tempera-**

**ture** 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.**

An NTSC video-rate camera reads the stored charge and outputs a video field every 16.7 milliseconds to conform to the recommended standards (denoted RS-170 or RS-330) in which 30 video frames are produced per second with each frame consisting of two interlaced fields (the European standard format requires 50 fields per second with a field every 20 milliseconds). **Video exploits the lag in our visual system by generating images at a rate faster than the critical flicker frequency, the video refresh frequency in which flicker is no longer perceived by the human eye.**

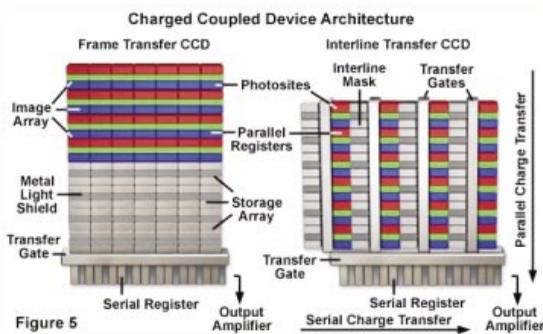
Each video field, containing 50 percent of the information in an entire frame, is obtained in sequence with the result that there is a 16.7 millisecond time difference between successive odd or even scan lines in the complete image. If the output of a video-rate camera is stopped and light is allowed to fall on the CCD for a prolonged period, the first two video fields produced contain all of the information accumulated during the integration period.

**Interlacing of two video fields to produce a complete video frame was a clever solution to the engineering problem resulting from the bandwidth limitations of the electronics and signal transmission and reception components available at the time of the development of television.**

Now much higher frequency amplifiers

and associated electronics permit the production, storage, and subsequent display at frame rates of up to 1000/second without the need to interlace scan lines. Such **progressive-scan** cameras produce a continuous scan from the top to the bottom of the image. This does not mean that the top lines were obtained before the bottom ones; rather, the devices first integrate the photon flux over the entire sensor and then rapidly displace the accumulated charge to a charge storage and transfer region that is protected from further illumination.

Two CCD designs are commonly used to achieve such rapid transfers: the **interline-transfer** CCD and the **frame-transfer** CCD, which are diagrammatically illustrated in Figure 5.



The **interline-transfer** CCD incorporates charge transfer channels (termed “interline masks” in Figure 5) 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.

The **spectral sensitivity of the CCD** differs from that of a simple silicon photodiode detector because the CCD surface has channels used for charge transfer that are **shielded** by polysilicon gate electrodes. These structures **absorb the shorter wavelengths and reduce the blue sensitivity of the device**. A typical spectral sensitivity curve for a consumer or scientific-grade CCD is illustrated in Figure 6 (Standard CCD) where it should be noted that the **peak quantum efficiency** of 40% is markedly **below** that of a individual silicon photodiode. Recently, the transparency of the channels has been increased with sub-

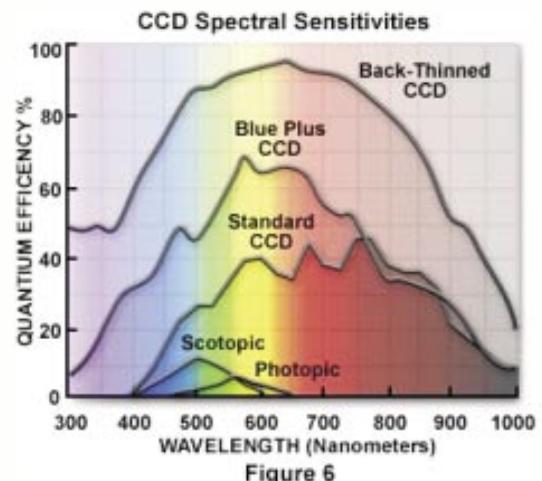


Figure 6

stantial **improvement in blue-green sensitivity** of some scientific-grade CCDs (Blue Plus curve in Figure 6).

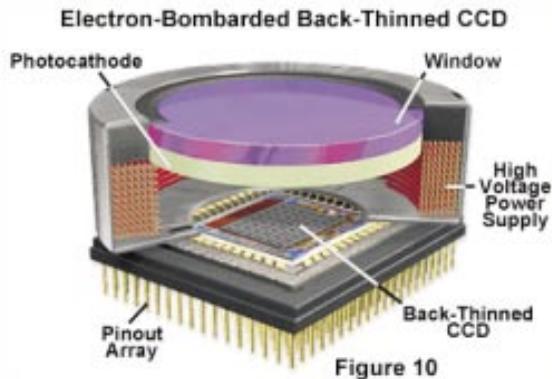


Figure 10

The losses due to the channels are completely **eliminated in the back-illuminated CCD**. In this design, light falls **onto the back of the CCD** in a region that has been thinned by etching until it is transparent (a thickness corresponding to about 10-15 microns). The resultant spectral sensitivity curve, also shown in Figure 6 (Back-Thinned CCD), illustrates the **high quantum efficiency** that can be realized with this configuration. However, back-thinning results in a **delicate, relatively expensive sensor** that, to date, has only been employed in high-end scientific-grade CCD cameras.

**The resolution of a CCD is a function of the number of photodiodes and their size relative to the projected image.**

CCD arrays of 1000 x 1000 sensors are now commonplace in **scientific-grade** video cameras. The trend in consumer and scientific-grade CCD manufacture is for the sensor size to decrease, and cameras with photodiodes as small as 4 x 4 microns are currently available in the consumer market. A typical **MTF curve** for a CCD camera with 6.7-micron pixels is shown in Figure 7. The spatial frequency of 60-percent contrast sine waves projected onto the sensor surface is plotted on the abscissa and the resultant modulation percentage on the ordinate. **The**

**limiting resolution is normally defined as the 3 percent modulation level.**

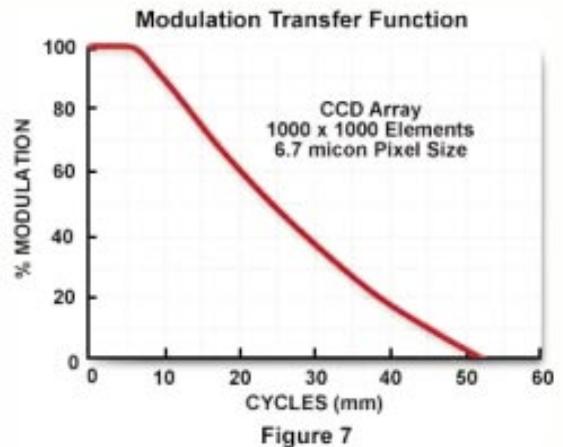


Figure 7

The **charge storage capacity** of a CCD is **proportional to the size of the individual photodiode**, such that the maximum number of electrons stored is about 1000 times the cross sectional area of each photodiode. Thus, a CCD with 7 x 7 micron photodiodes should have a maximum charge storage capacity (a full-well capacity) of 49,000 **electrons or holes**. A hole is the region of the silicon from which the electron came and constitutes an equally valid and usable measure of detected photons. The term “electrons” will be used predominantly throughout this discussion even though many CCDs read out the number of holes generated rather than electrons. Because **CCDs do not have inherent gain, one electron-hole pair is accumulated for each detected photon.**

The **dynamic range** of a CCD is typically defined as **the full-well capacity divided by the camera noise**. The camera noise is the sum, in quadrature, of the dark and read-out noise. **Recent improvements in CCD design have greatly diminished dark charge to negligible levels and reduced read-out noise to about 10 electrons per pixel.** Even room temperature cameras may have such a low dark signal that it can be ignored for **integration peri-**

ods of 10 seconds or less. Cooling further reduces the dark signal and permits much longer integration periods, up to several hours, without significant dark charge accumulation.

Thus, the dynamic range of a 49,000 electron full-well capacity CCD with 10 electrons of read-out noise and negligible dark noise is about 4900, **corresponding to a 12-bit dynamic range** (4096 gray-scale levels).

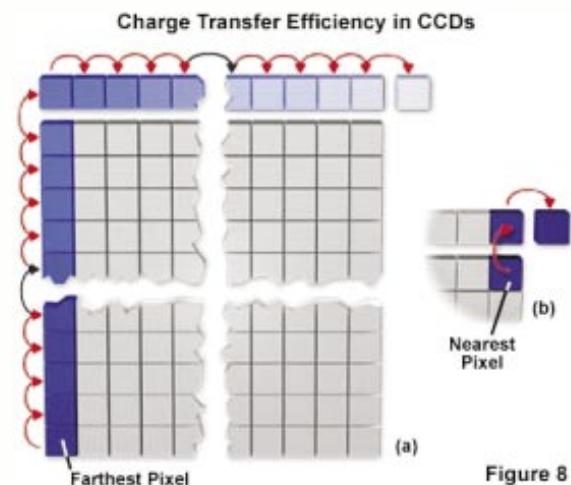
A CCD with a 49,000 electron full-well capacity has a maximum achievable signal/noise of about 220 (the square root of 49,000). Of course, camera noise would add, in quadrature, to the photon statistical noise and reduce the maximum S/N below this value. A simple estimate of the S/N of any homogeneous region in an image may be made from the average intensity of the region of interest divided by the standard deviation of the intensities of that region.

Cameras for consumer use often have a rectangular format CCD with an aspect ratio of 4:3. That means that the height of the image will be 3/4 of the width to conform with video standards based on our landscape view of the world. Indeed, the newest generation of consumer-grade products designed for HDTV employ a 16:9 aspect ratio. **Scientific imaging**, on the other hand, is **best conducted with a square image** made up of **square pixels** as they are better suited to digital image processing.

**CCD sensor uniformity is generally very good, with less than 10 percent variation in gain between photodiodes.** However, **shading** may be introduced into the image from a CCD camera because of **inefficiencies in charge transfer**.

The operation of a CCD requires that each packet of charge underlying a photodiode be transferred to the read-out amplifier.

This transfer is accomplished by a **series of parallel and serial shifts** that displace rows of charge along the chip toward a single corner containing the read-out amplifier. If the read-out amplifier is in the upper right-hand corner of a 1000 x 1000 sensor CCD, the charge from the photosensor nearest to that corner will have to be shifted only once upward into the serial shift register (a parallel shift) and once rightward (a serial shift) to reach the amplifier. On the other hand, the charge from the photodiode in the lower left hand corner will have to be shifted upward 1000 times and rightward 1000 times to be read out. If the transfer efficiency is 99.9 percent for each shift, only 13.5 percent of the charge accumulated by the lower left photodiode would remain after the requisite



2000 shifts. This charge loss would make the lower left corner much darker than the upper right and would also tend to blur or smear that region of the image because of spillover by charges from adjacent photodiodes. The concept is illustrated schematically in Figure 8 using blue colored pixels to represent integrated charge density. The pixel in the lower left hand corner of the CCD (farthest pixel - dark blue) is shown slowly losing color intensity as the charge is transferred first in parallel to the serial shift register, then serially to the output node. A pixel closer to the node (the upper right-

hand “nearest” pixel) is also illustrated in dark blue and undergoes only a two-step jump to yield an accumulated charge transfer efficiency of 99.8 percent at the output node.

Slow-scan CCD cameras increase charge transfer efficiency by **cooling the CCD** and slowing the transfer rate. The high speed charge transfer required in video-rate CCD cameras necessitates a different strategy. In these cameras, the read-out amplifier gain is adjusted to compensate for the charge lost from each row by sampling extra pixels outside of the image area. The additional gain required for the lower rows inevitably increases the noise in the highly corrected regions of the sensor. Some control over the read-out rate as well as the size of the pixel that constitutes a sensor is permitted by slow-scan CCD cameras. **Video-rate CCD cameras are simpler** and do not allow such control. Slowing the read-out usually reduces the amplifier noise associated with reading the charge, a beneficial situation when the photon flux is very low and the signal can be produced relatively slowly (in a second or two rather than in 33 milliseconds). **Scientific-grade CCD cameras usually offer two or more read-out rates so that speed may be traded off against noise.**

The size of a pixel in a scientific-grade CCD may be increased by **binning**, a process in which the charge from a cluster of adjacent photodiodes is pooled and **treated as if it came from a larger detector**. In binning, several shifts of charge to the serial register and output node storage regions occur before read out. The extent of binning depends on how many shifts occur before the stored charge is read, with the only limitation being the charge storage capacity of the serial register (usually twice that of a single photodiode) or of the output node

(usually three times that of a photodiode). The maximum charge storage capacity of the serial register and output node are not a concern in most microscopy applications because binning is employed when light levels are very low and few photons are detected. Binning enables the investigator to trade spatial resolution for sensitivity.

### Electronic versus Visual Detection

How does the human eye compare with electronic detectors?

Figure 6 illustrates spectral sensitivity curves for the eye, corresponding to photopic and scotopic vision, arising from the cones and rods (Figure 12), respectively. **Peak sensitivity is in the green** (photopic at 555 nanometers and scotopic at 507 nanometers) with a maximum quantum efficiency of 3 percent for photopic vision and 10 percent for scotopic. **Our spatial resolution is not uniform because the cones are not evenly distributed.**

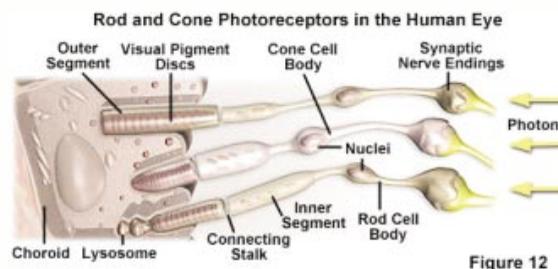


Figure 12

**The highest density occurs in the fovea where the distance between cones is about 1.5 microns, giving us a 5 to 6 micron limiting spatial resolution on the retina.**

Under **achromatic** (black and white) constant illumination conditions, **visual intrascene dynamic range is only about 50-fold (6 bits)**. Our visual pigment, **rhodopsin**, exhibits little thermal noise, and the minimum detectable signal after dark adaptation is about 100 to 150 photons at

the pupil or about 10 to 15 photons at the retina.

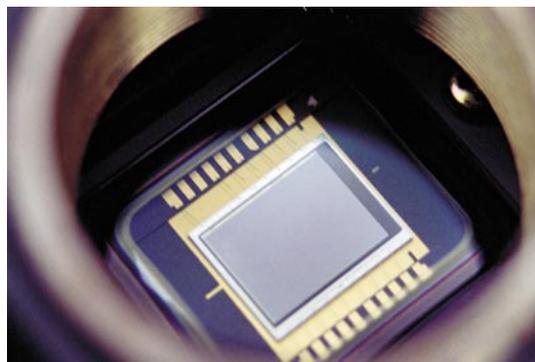
**The signal/noise for the eye at the visual detection limit is about 3:1. Lag (Persistence) is about 20 milliseconds at high light levels and about 100 milliseconds in dim illumination.**

**It is obvious that, compared to our eyes, a scientific-grade CCD camera has a broader spectral sensitivity, much higher quantum efficiency, greater integration capability, more uniformity, better intrascene dynamic range (more “bits”), comparable or higher signal/noise, but lower spatial resolution.**

When matched against our visual system, low-light-level cameras have a much wider spectral range, less lag and far greater sensitivity and resolution under photon-limited conditions.

### Choosing the Appropriate Camera

Two types of color CCD cameras are used for scientific applications: a **single CCD** with a wavelength selection filter or a three sensor (**three chip**) camera. Both use filters to produce red, green and blue versions of the field-of-view. The single sensor camera utilizes an **adherent filter**, filter wheel or liquid-crystal tunable filter to acquire the red, green, and blue images. The three sensor camera has a **beam splitting prism** and trim filters that enable each sensor to image the appropriate color and to acquire all three images simultaneously. Invariably, **color cameras are less sensitive than their monochrome counterparts** because of the additional beam-splitting and wavelength



selection components. In some applications, the loss of sensitivity is offset by being able to capture multiple wavelengths simultaneously. In addition, some color cameras achieve a higher resolution by employing a piezo-controlled translocation mechanism to offset the CCD slightly, thereby increasing the sampling frequency.

Recent improvements in the performance of CMOS cameras herald a potentially **important future role** for these devices. **CMOS cameras have an amplifier and digitizer associated with each photodiode in an integrated on-chip format.** The individual amplifiers associated with each pixel help reduce noise and distortion levels, but they also **induce** an artifact known as “**fixed pattern noise**” that arises from switching and sampling artifacts of individual pixel amplifiers. This is manifested by reproducible patterns of “mottle” behavior in the image generated by CMOS active pixel sensor devices. A great deal of research effort has been invested in solving this problem, and the residual level of noise has recently been dramatically reduced in CMOS sensors. The result is a **cheap, compact, versatile detector** combining the virtues of silicon detection without the problems of charge transfer. CMOS sensors allow gain manipulation of individual photodiodes, region-of-interest read-out, high speed sampling, electronic shuttering and exposure control. They have extraordinary dynamic range as well as

an ideal format for the computer interface. It is likely that they will replace CCD cameras in a number of scientific applications in the near future.

*Source: <http://microscopy.fsu.edu>*