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## Converting Light to an Electronic Charge

(Kodak)

An image is acquired when incident light in the form of photons falls on the array of pixels. The energy associated with each photon is absorbed by the silicon and a reaction takes place that creates an electron-hole charge pair (for example, an electron). The number of electrons collected at each pixel is linearly dependent on light level and exposure time, nonlinearly dependent on wavelength.

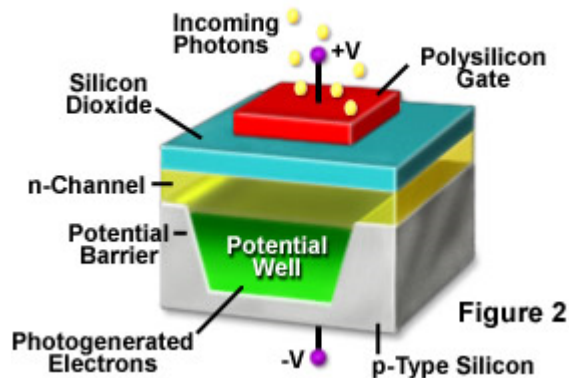
(Nikon, <http://www.microscopyu.com/articles/digitalimaging/ccdintro.html>)

The light-sensing photodiode elements of the CCD respond to incident photons by absorbing much of their energy, resulting in liberation of electrons, and the formation of corresponding electron-deficient sites (holes) within the silicon crystal lattice. One electron-hole pair is generated from each absorbed photon, and the resulting charge that accumulates in each pixel is linearly proportional to the number of incident photons. External voltages applied to each pixel's electrodes control the storage and movement of charges accumulated during a specified time interval. Initially, each pixel in the sensor array functions as a **potential well** to store the charge during collection, and although either negatively charged electrons or positively charged holes can be accumulated (depending on the CCD design), the charge entities generated by incident light are usually referred to as **photoelectrons**. This discussion considers electrons to be the charge carriers. These photoelectrons can be accumulated and stored for long periods of time before being read from the chip by the camera electronics as one stage of the imaging process.

Image generation with a CCD camera can be divided into four primary stages or functions: charge generation through photon interaction with the device's photosensitive region, collection and storage of the liberated charge, charge transfer, and charge measurement. During the first stage, electrons and holes are generated in response to incident photons in the depletion region of the MOS capacitor structure, and liberated electrons migrate into a potential well formed beneath an adjacent positively-biased gate electrode. The system of aluminum or polysilicon surface gate electrodes overlies, but are separated from, charge carrying channels that are buried within a layer of insulating silicon dioxide placed between the gate structure and the silicon substrate. Utilization of polysilicon as an electrode material provides transparency to incident wavelengths longer than approximately 400 nanometers and increases the proportion of surface area of the device that is available for light collection. Electrons generated in the depletion region are initially collected into electrically positive potential wells associated with each pixel. During readout, the collected charge is subsequently shifted along the transfer channels under the influence of voltages applied to the

gate structure. Figure 3 illustrates the electrode structure defining an individual CCD sense element.

### Metal Oxide Semiconductor (MOS) Capacitor



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Digital image quality can be assessed in terms of four quantifiable criteria that are determined in part by the CCD design, but which also reflect the implementation of the previously described camera operation variables that directly affect the imaging performance of the CCD detector. The principal image quality criteria and their effects are summarized as follows:

- **Spatial Resolution:** Determines the ability to capture fine specimen details without pixels being visible in the image.
- **Light-Intensity Resolution:** Defines the dynamic range or number of gray levels that are distinguishable in the displayed image.
- **Time Resolution:** The sampling (frame) rate determines the ability to follow live specimen movement or rapid kinetic processes.
- **Signal-to-Noise Ratio:** Determines the visibility and clarity of specimen signals relative to the image background.

### CCD Camera Noise Sources

Camera sensitivity, in terms of the minimum detectable signal, is determined by both the photon statistical (shot) noise and electronic noise arising in the CCD. A conservative estimation is that a signal can only be discriminated from accompanying noise if it exceeds the noise by a factor of approximately 2.7 (SNR of 2.7). The minimum signal that can theoretically yield a given SNR value is determined by random variations of the photon flux, an inherent noise source associated with the signal, even with an ideal noiseless detector. This photon statistical noise is equal to the square root of the number of signal photons, and since it cannot be eliminated, it determines the maximum achievable SNR for a noise-free detector. The signal/noise ratio is therefore given by the signal level,  $S$ , divided by the square-root of the signal ( $S(1/2)$ ), and is equal to the square-root of  $S$ . If a SNR value of 2.7 is required for discriminating signal from noise, a signal level of 8 photons is the minimum theoretically detectable light flux.

In practice, other noise components, which are not associated with the specimen photon signal, are contributed by the CCD and camera system electronics, and add to the inherent photon statistical noise. Once accumulated in collection wells, charge arising from noise sources cannot be distinguished from photon-derived signal. Most of the **system noise** results from readout amplifier noise and thermal electron generation in the silicon of the detector chip. The thermal noise is attributable to kinetic vibrations of silicon atoms in the CCD substrate that liberate electrons or holes even when the device is in total darkness, and which subsequently accumulate in the potential wells. For this reason, the noise is referred to as **dark noise**, and represents the uncertainty in the magnitude of dark charge accumulation during a specified time interval. The rate of generation of dark charge, termed **dark current**, is unrelated to photon-induced signal but is highly temperature dependent. In similarity to photon noise, dark noise follows a statistical (square-root) relationship to dark current, and therefore it cannot simply be subtracted from the signal. Cooling the CCD reduces dark charge accumulation by an order of magnitude for every 20-degree Celsius temperature decrease, and high-performance cameras are usually cooled during use. Cooling even to 0 degrees is highly advantageous, and at -30 degrees, dark noise is reduced to a negligible value for nearly any microscopy application.

Providing that the CCD is cooled, the remaining major electronic noise component is **read noise**, primarily originating with the on-chip preamplifier during the process of converting charge carriers into a voltage signal. Although the read noise is added uniformly to every pixel of the detector, its magnitude cannot be precisely determined, but only approximated by an average value, in units of electrons (root-mean-square or rms) per pixel. Some types of readout amplifier noise are frequency dependent, and in general, read noise increases with the speed of measurement of the charge in each pixel. The increase in noise at high readout and frame rates is partially a result of the greater amplifier bandwidth required at higher pixel clock rates. Cooling the CCD reduces the readout amplifier noise to some extent, although not to an insignificant level. A number of design enhancements are incorporated in current high-performance camera systems that greatly reduce the significance of read noise, however. One strategy for achieving high readout and frame rates without increasing noise is to electrically divide the CCD into two or more segments in order to shift charge in the parallel register toward multiple output amplifiers located at opposite edges or corners of the chip. This procedure allows charge to be read out from the array at a greater overall speed without excessively increasing the read rate (and noise) of the individual amplifiers.

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## **Spatial and Temporal Resolution in CCD Image Sensors**

In many applications, an image capture system capable of providing high **temporal resolution** is a primary requirement. For example, if the kinetics of a process being studied necessitates video-rate imaging at moderate resolution, a camera capable of delivering superb resolution is, nevertheless, of no benefit if it only provides that performance at slow-scan rates, and performs marginally or not at all at high frame rates. Full-frame slow-scan cameras do not deliver high resolution at video rates, requiring approximately one second per frame for a large pixel array, depending upon the digitization rate of the electronics. If specimen signal brightness is sufficiently high to allow short exposure times (on the order of 10 milliseconds), the use of binning and subarray selection makes it possible to acquire about 10 frames per second at reduced resolution and frame size with cameras having electromechanical shutters. Faster frame rates generally necessitate the use of interline-

transfer or frame-transfer cameras, which do not require shutters and typically can also operate at higher digitization rates. The latest generation of high-performance cameras of this design can capture full-frame 12-bit images at near video rates.

The now-excellent **spatial resolution** of CCD imaging systems is coupled directly to pixel size, and has improved consistently due to technological improvements that have allowed CCD pixels to be made increasingly smaller while maintaining other performance characteristics of the imagers. In comparison to typical film grain sizes (approximately 10 micrometers), the pixels of many CCD cameras employed in biological microscopy are smaller and provide more than adequate resolution when coupled with commonly used high-magnification objectives that project relatively large-radii diffraction (Airy) disks onto the CCD surface. Interline-transfer scientific-grade CCD cameras are now available having pixels smaller than 5 micrometers, making them suitable for high-resolution imaging even with low-magnification objectives. The relationship of detector element size to relevant optical resolution criteria is an important consideration in choosing a digital camera if the spatial resolution of the optical system is to be maintained.

The Nyquist sampling criterion is commonly utilized to determine the adequacy of detector pixel size with regard to the resolution capabilities of the microscope optics. Nyquist's theorem specifies that the smallest diffraction disk radius produced by the optical system must be sampled by at least two pixels in the imaging array in order to preserve the optical resolution and avoid aliasing. As an example, consider a CCD having pixel dimensions of 6.8 x 6.8 micrometers, coupled with a 100x, 1.3 numerical aperture objective, which produces a 26-micrometer (radius) diffraction spot at the plane of the detector. Excellent resolution is possible with this detector-objective combination, because the diffraction disk radius covers approximately a 4-pixel span ( $26 / 6.8 = 3.8$  pixels) on the detector array, or nearly twice the Nyquist limiting criterion. At this sampling frequency, sufficient margin is available that the Nyquist criterion is nearly satisfied even with 2 x 2 pixel binning.

### Image Sensor Quantum Efficiency

Detector **quantum efficiency (QE)** is a measure of the likelihood that a photon having a particular wavelength will be captured in the active region of the device to enable liberation of charge carriers. The parameter represents the effectiveness of a CCD imager in generating charge from incident photons, and is therefore a major determinant of the minimum detectable signal for a camera system, particularly when performing low-light-level imaging. No charge is generated if a photon never reaches the semiconductor depletion layer or if it passes completely through without transfer of significant energy. The nature of interaction between a photon and the detector depends upon the photon's energy and corresponding wavelength, and is directly related to the detector's **spectral sensitivity range**. Although conventional front-illuminated CCD detectors are highly sensitive and efficient, none have 100-percent quantum efficiencies at any wavelength.

Image sensors typically employed in fluorescence microscopy can detect photons within the spectral range of 400-1100 nanometers, with peak sensitivity normally in the range of 550-800 nanometers. Maximum QE values are only about 40-50 percent, except in the newest designs, which may reach 80 percent efficiency. Figure 10 illustrates the spectral sensitivity of a number of popular CCDs in a graph that plots quantum efficiency as a function of incident light wavelength. Most CCDs used in scientific imaging are of the interline-transfer type, and because the interline mask severely limits the photosensitive surface area, many

older versions exhibit very low QE values. With the advent of the surface microlens technology to direct more incident light to the photosensitive regions between transfer channels, newer interline sensors are much more efficient and many have quantum efficiency values of 60-70 percent.

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## Dynamic Range

A term referred to as the **dynamic range** of a CCD detector expresses the maximum signal intensity variation that can be quantified by the sensor. The quantity is specified numerically by most CCD camera manufacturers as the ratio of pixel full well capacity (**FWC**) to the read noise, with the rationale that this value represents the limiting condition in which intrascene brightness ranges from regions that are just at pixel saturation level to regions that are barely lost in noise. The sensor dynamic range determines the maximum number of resolvable gray-level steps into which the detected signal can be divided. To take full advantage of a CCD's dynamic range, it is appropriate to match the analog-to-digital converter's bit depth to the dynamic range in order to allow discrimination of as many gray scale steps as possible. For example, a camera with a 16,000-electron FWC and readout noise of 10 electrons, has a dynamic range of 1600, which supports between 10 and 11-bit A/D conversion. Analog-to-digital converters with bit depths of 10 and 11 are capable of discriminating 1024 and 2048 gray levels, respectively. As stated previously, because a computer bit can only assume one of two possible states, the number of intensity steps that can be encoded by a digital processor (ADC) reflects its resolution (bit depth), and is equal to 2 raised to the value of the bit depth specification. Therefore, 8, 10, 12, and 14-bit processors can encode a maximum of 256, 1024, 4096, or 16384 gray levels.

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**Shot noise** is a type of [electronic noise](#) that occurs when the finite number of particles that carry energy, such as [electrons](#) in an electronic circuit or [photons](#) in an optical device, is small enough to give rise to detectable statistical fluctuations in a measurement. It is important in [electronics](#), [telecommunications](#), and fundamental [physics](#).

It also refers to an analogous noise in particle simulations, where due to the small number of particles, the simulation exhibits detectable statistical fluctuations not observed in the real-world system.

The magnitude of this noise increases with the average magnitude of the current or intensity of the light. However, since the magnitude of the average signal increases more rapidly than that of the shot noise (its relative strength decreases with increasing signal), shot noise is often only a problem with small currents or light intensities.

The intensity of a source will yield the *average* number of photons collected, but knowing the average number of photons which will be collected will not give the actual number collected.

The actual number collected will be more than, equal to, or less than the average, and their distribution about that average will be a [Poisson distribution](#).

Since the Poisson distribution approaches a [normal distribution](#) for large numbers, the photon noise in a signal will approach a normal distribution for large numbers of photons collected. The [standard deviation](#) of the photon noise is equal to the square root of the average number of photons. The [signal-to-noise ratio](#) is then

$$\text{SNR} = \frac{N}{\sqrt{N}} = \sqrt{N}$$

where  $N$  is the average number of photons collected. When  $N$  is very large, the signal-to-noise ratio is very large as well. It can be seen that photon noise becomes more important when the number of photons collected is small.

SNR computation demo

<http://www.microscopyu.com/tutorials/java/digitalimaging/signaltonoise/>