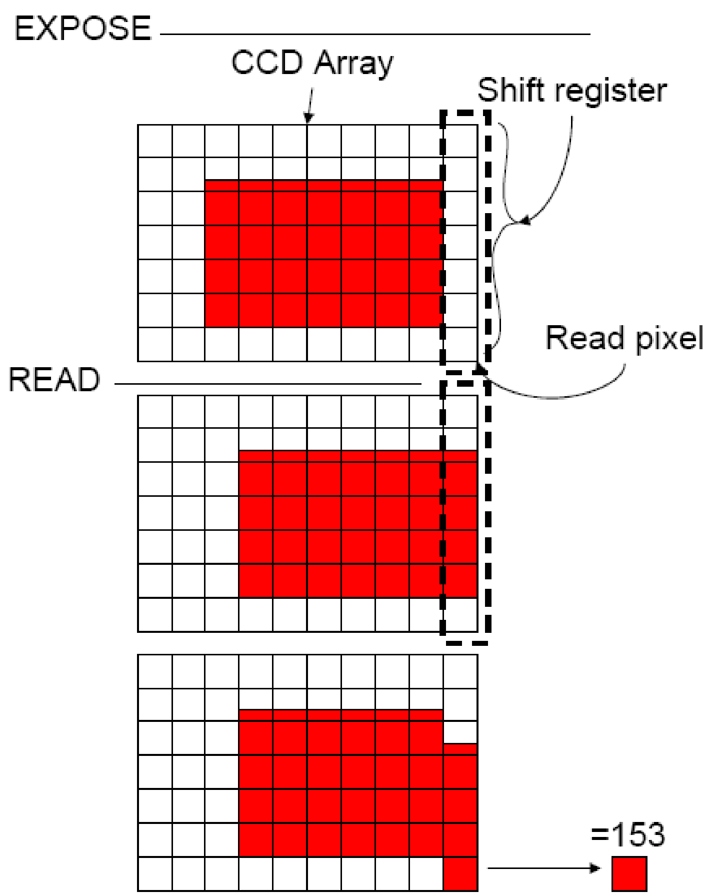
A brief primer on digital imaging (by DW)**:**
4 sections:
1) basics of CCDs. 2) glossary of terms. 3) how to image. 4) software help

The basics of CCDs (charge coupled devices):
In Aph162, we use CCDs to capture an image. How does a CCD work? We can think of a CCD as an array of pixels, each of which can hold a set number of electrons; the electrons are generated when a photon arrives at the pixel. Since a CCD is an array of pixels, we can form an image. That being said, there are a few basic parameters we need to take into consideration when forming our image. First of all is noise: the detectors in this lab are not single-photon imaging devices since the inherent (statistical) noise of the system limits the signal to noise ratio. Additional sources of noise arise when we convert this inherently analog signal to digital form (read noise). However, if we integrate over long times (or increase the brightness of our samples), we can usually reduce statistical noise, but not read noise, since the A to D conversion takes place after we have formed our image. After exposing an image on the CCD for some time, the pixels are traditionally (as of late 1990's, at least) shifted in one direction to a register on the edge of the chip, before being read out one pixel at a time (see figure). This should give you an idea of what we need to do in order to form a nice image.



Caption: An image is exposed onto the CCD. After exposure, all the pixels are shifted in the direction of the register. The pixels are then read out individually. When talking about "speed of the chip", given in frequency units, is the speed at which each individual pixel is converted to a number. Assuming performing shifting operations is extremely fast (clock speed), then, the amount of time it takes to read out an image (before the next image is exposed) is limited by the speed of the chip. Thus for a megapixel CCD running at 1 MHz speed, it takes a second to convert an image to digital storage form. This is the reason most digital cameras cannot take such fast, continuous exposures. Naturally, there are other types and ways by which CCD images are processed (such as, for instance, shifting an entire image (called frame-transfer), not just a row at a time.). The figure describes a basic way.

Glossary of terms:

Shot noise: (photon noise) electrons generated when photons arrive at the detector fluctuate according to the square root of number of photons, since the photoelectron arrival obeys Poisson statistics. The spatial correlation of this type of noise does not extend beyond one pixel, and hence can be filtered after the fact with an appropriate high frequency bandpass filter.

Dark noise: also a form of statistical noise, this refers to the phenomenon that working at finite temperatures, electrons are thermally generated, resulting in a constant background of noise. One can overcome this by lowering the temperature of the CCD chip.

Spatial noise: Constant spatial variations in the sensitivity of pixels on the chip, perhaps due to inconsistent manufacturing or smudges on optics somewhere. This type of noise can be background subtracted.

Clock noise: Shifting pixels into the register is timed based on a clock, which generates noise. There is an associated clock speed, naturally.

Read noise: The noise of analog to digital conversion.

Read frequency: The speed at which each pixel is read out, approximately. This is usually fixed, but some cameras have variable speeds. Increasing the speed will increase the amount of read noise.

Exposure time: the amount of time light falls on the CCD.

Dynamic range: The depth of each pixel, in terms of ability to store electrons: usually 8-bit or 12-bit or whatever. This limits the range of information available in each image.

Electronic shuttering: whenever light is applied to a CCD, electrons are generated. This electronically controlled shutter blocks light to the CCD.

Gain: After reading values (after A-D conversion), the signal is multiplied by certain numerical factors. Gain does not reduce read noise, the largest source of noise on any given camera. (Thus, technologies have been developed to put the gain on-chip (electron-multiplying CCDs) or to amplify the amount of light hitting the sensor (intensified CCDs).)

Blooming: If the signal saturates the dynamic range, current can still flow but the storage capacity is done for. Thus signal may spill to adjacent pixels, producing artifacts.

Binning: In which multiple pixels are considered as one. Gets rid of shot noise.

Quantum efficiency: The sensitivity of the CCD to certain wavelengths of light, and how often a photon of light is converted to an electron. Typical values are less than 30%.

How to image:
In order for us to see something, the size of our object should be bigger than the spatial correlation of shot noise (1 pixel) on a CCD, and the signal should be greater than noise. In order to achieve this, we need to think of the appropriate magnification, strength of the signal, and exposure times on chip. One must think carefully, especially if moving objects are to be captured electronically. Furthermore, we wish to have lots of sensitivity and dynamic range for post processing and image analysis: for instance, for finding where the area of most rapid change in signal occurs. A signal with small dynamic range would not accurately capture steep changes in intensity.

Software protocols: (under development)

Zeiss (Axiocam):

Slidebook: This is the most confusing, since the microscope is a mixture of automated and manual controls. One key thing is that the manual filter wheel should be in register with the software, and that the software controls an excitation filter wheel.

MoticCam

Other