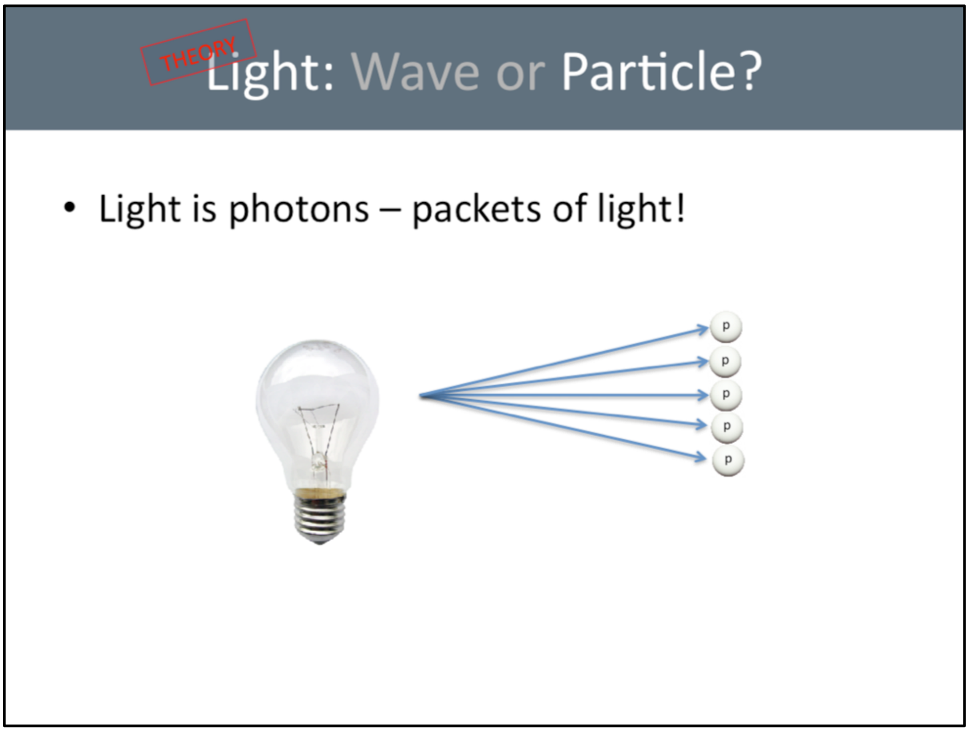
Image Capture (and Manipulation)

January 2013

Image Capture and Manipulation - Overview

- Light detectors come in 2 flavors
  - CCD cameras – on fluorescence microscopes in Dunn School
  - PMT detectors (on confocals)

- Images are just an array of photon counts
- Images can be manipulated to appear different – ie brightness, contrast.



Light is Photons – Little packets of Light

We've seen in earlier lectures that Light is part of the Electro-magnetic spectrum. One consequence of this is that it can be thought of as having 'wave-like' properties. However another consequence is that Light can **<u>also</u>** be thought of as being packets of light called photons. This duality is a consequence of quantum theory and is outside the remit of this lecture.

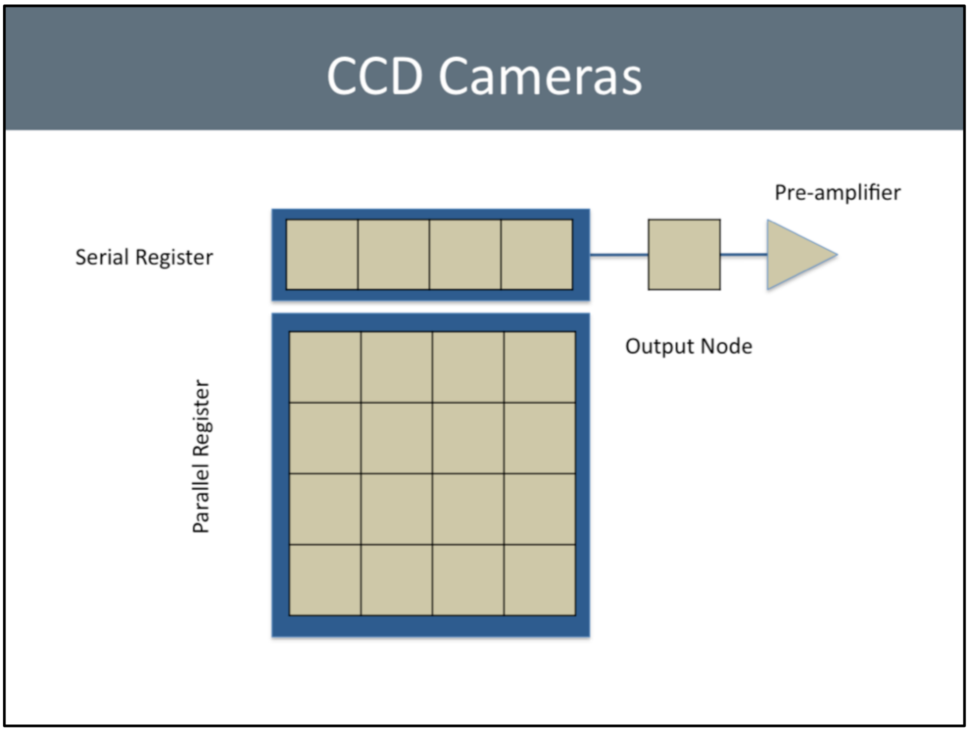
However when it comes to image capture it is useful to think of light as being made of discrete packets.

CCD Cameras

Invented in 1970 at Bell Labs
• A silicon chip that converts an image to an electrical signal
• Image is focused directly onto the silicon chip
• Widely used in TV cameras and consumer camcorders

CCD – Charge Coupled Device cameras

Until recently these were the basis of all digital camera devices.
Made from a single sheet of silicon separated into an array of photo-reactive ‘cells’ which convert photons into an electrical charge. This charge can be passed across these cells in a control fashion.



CCD camera layout

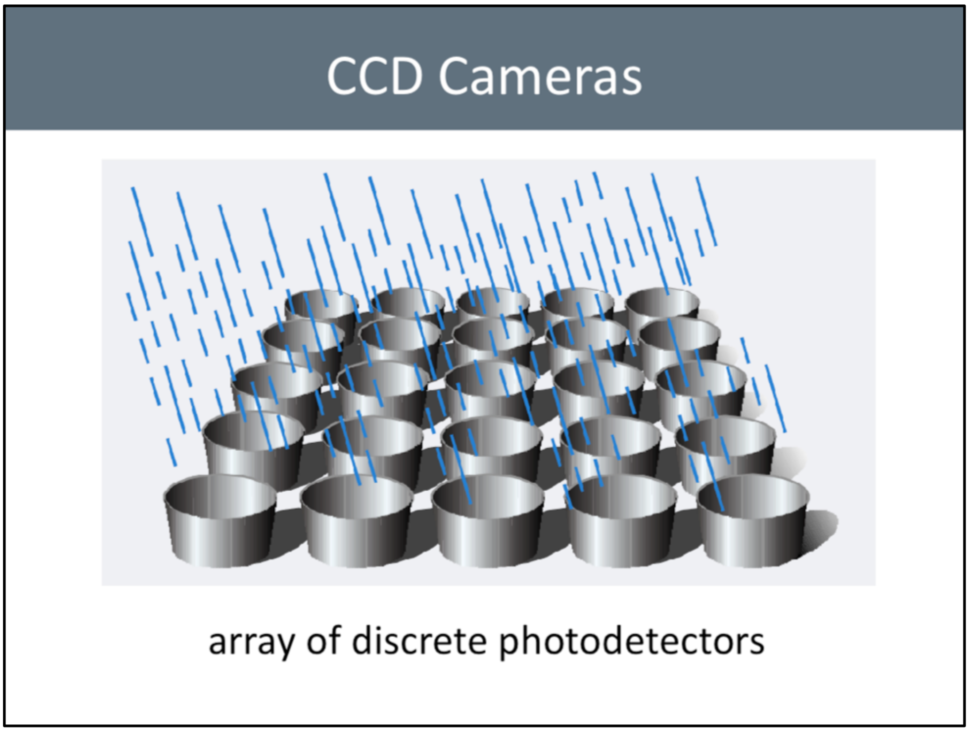
For the purpose of this lecture a CCD camera is composed of 4 elements:

A parallel register – this is the silicon chip divided into photo reactive 'cells'. The image is focused onto this surface.

a serial register – onto which the electrons are passed

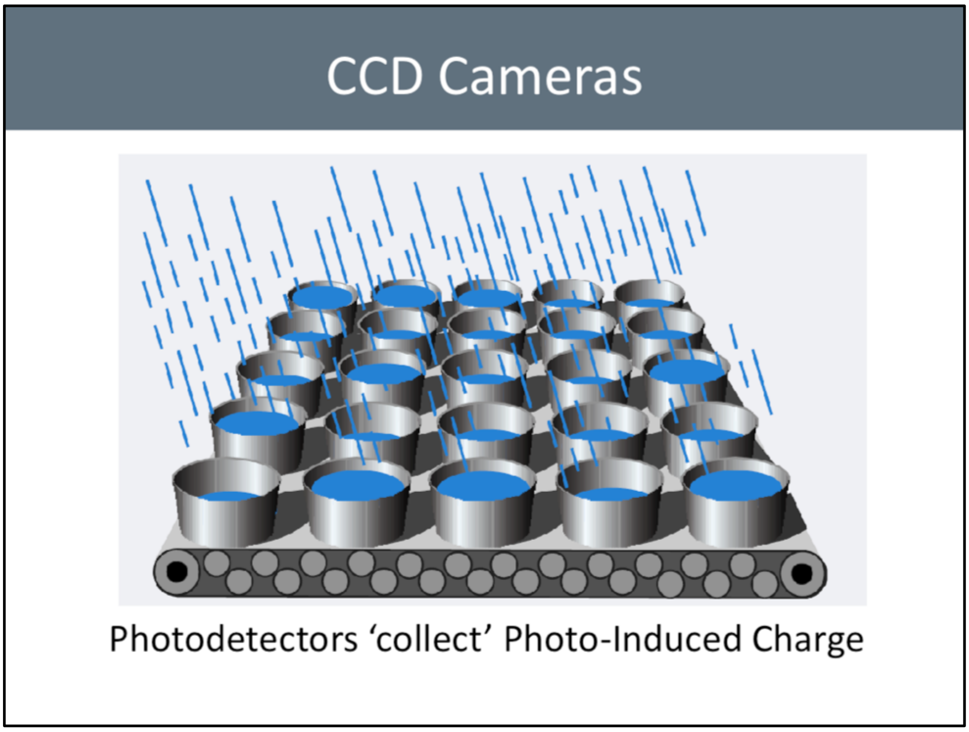
an output node – where electrons are passed 'one cell at a time'

a pre-amplifier – which send the electron counts from the output node through to an attached computer which then processes the reconstitutes the electron counts into an image.



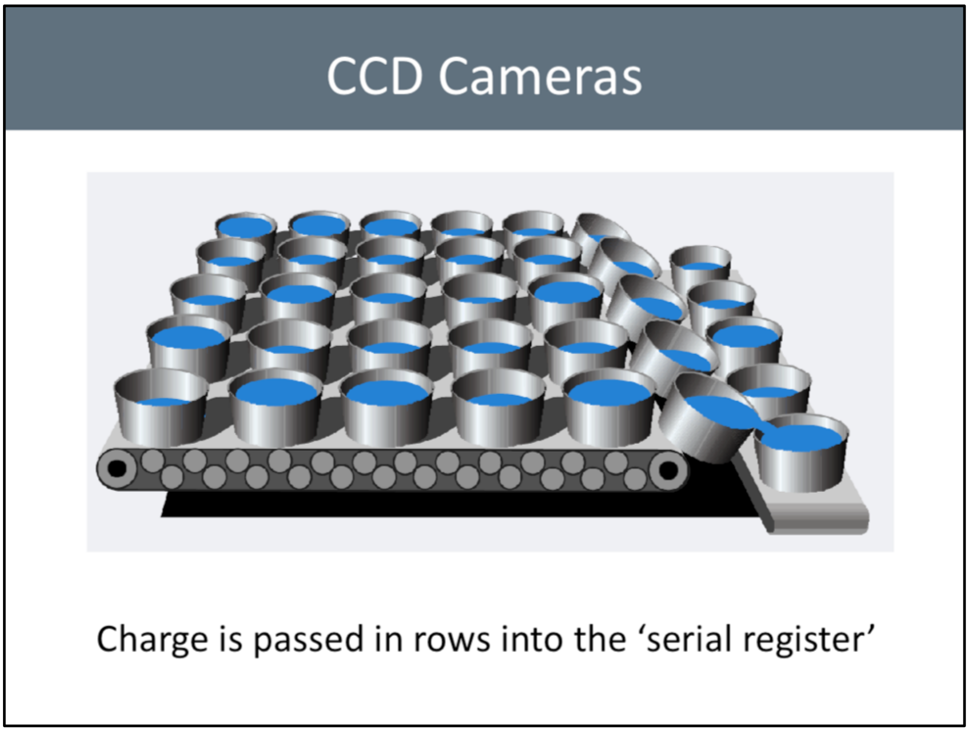
CCD Cameras

CCD cameras can be thought of as an array of buckets (the photo-reactive cells) collecting rainwater (photons)



CCD Cameras

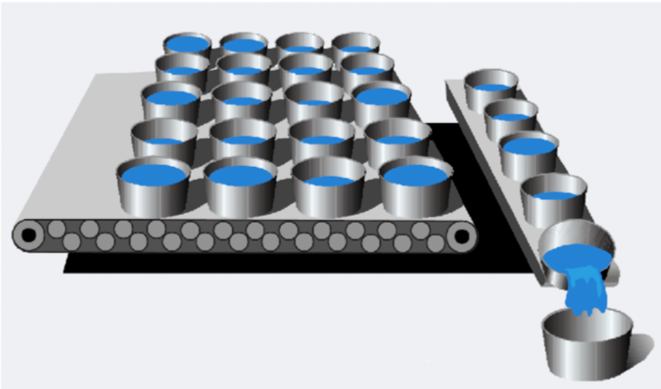
The buckets (the photo-reactive cells) collect rainwater (photons) for a certain period of time (length of exposure).



CCD Cameras

After which the buckets are emptied out row by row into a serial row of buckets (the serial register)

CCD Cameras

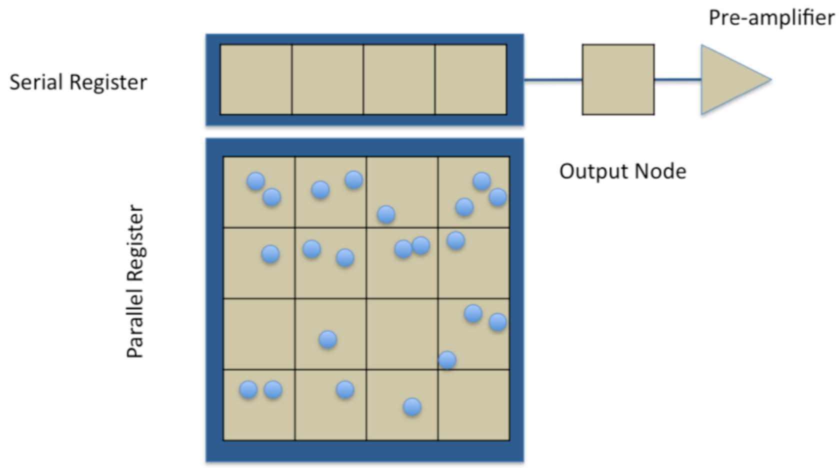


Charge in ‘serial register’ is passed one-by one into output node

CCD Cameras

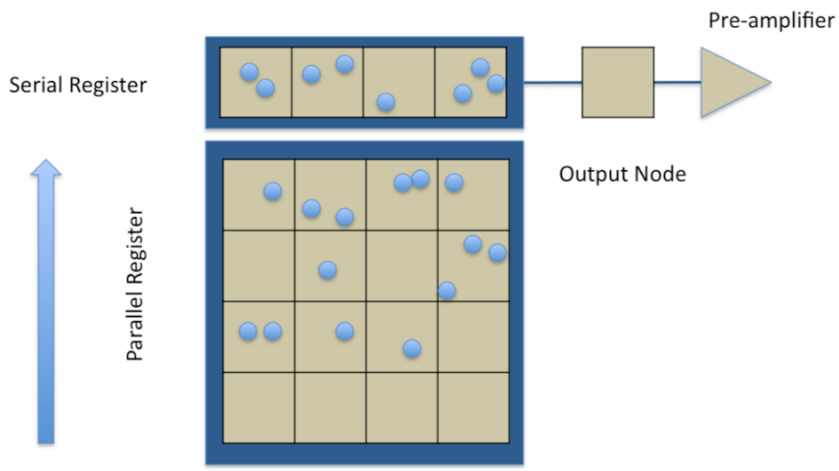
Rainwater (photons) is then passed into one special bucket (the output node) where an observing weatherman (the pre-amp) measure how full the bucket is and then tells his friend (the computer!)

CCD Cameras

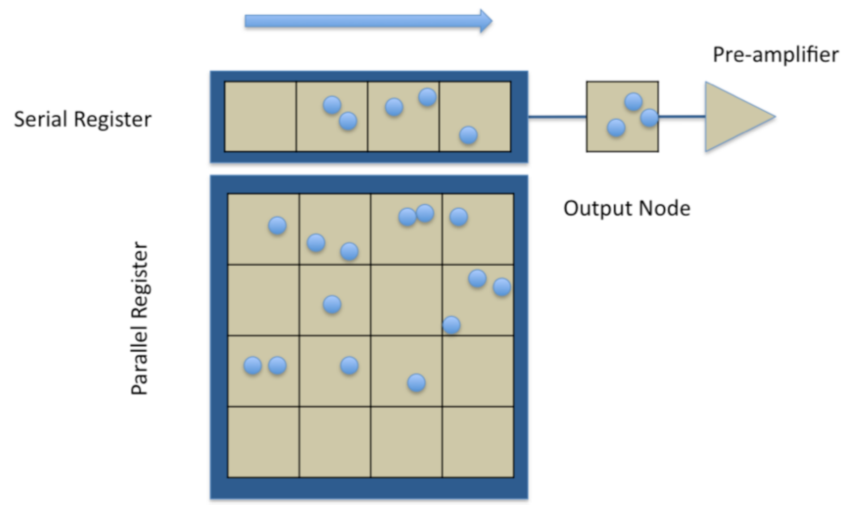


Pre-amplifier
Serial Register
Output Node
Parallel Register

CCD Cameras

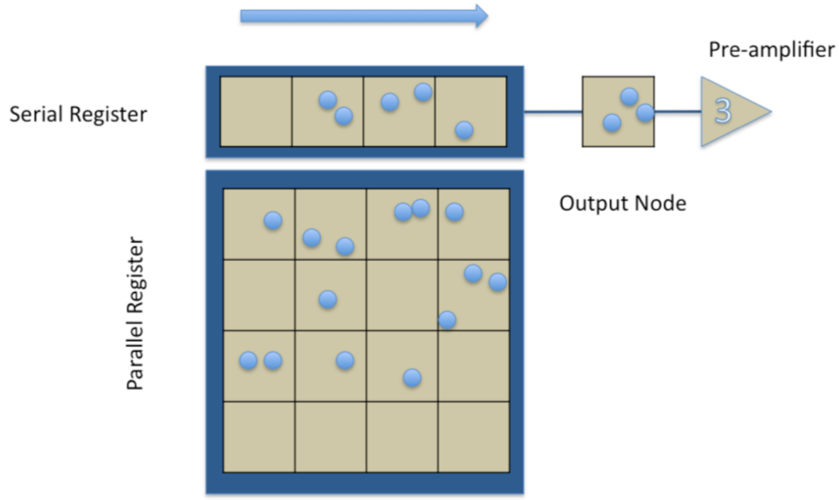

Pre-amplifier
Serial Register
Output Node
Parallel Register

CCD Cameras

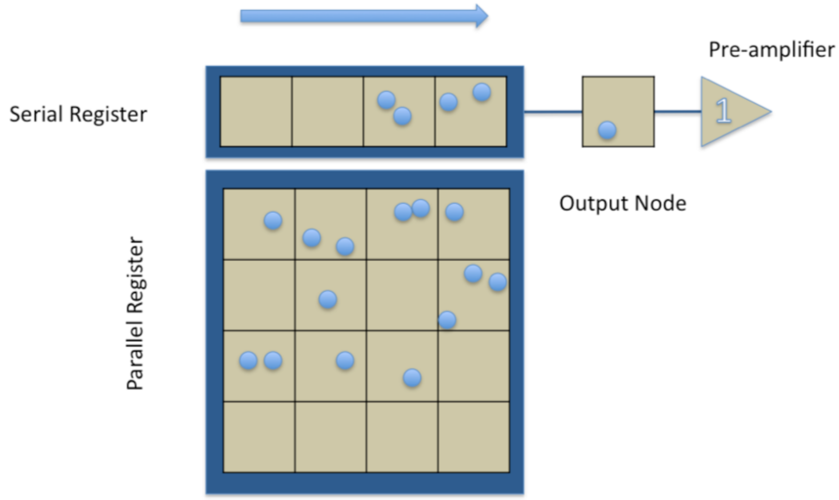


Pre-amplifier
Serial Register
Output Node
Parallel Register

CCD Cameras

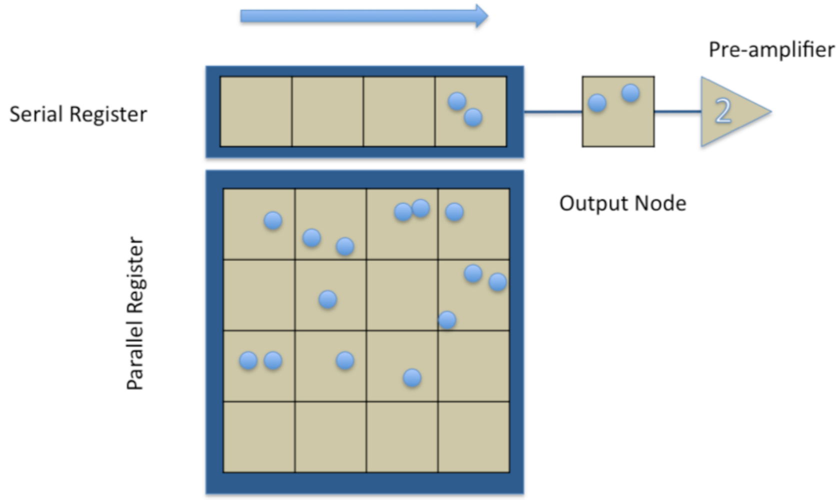

Pre-amplifier
Serial Register
3
Output Node
Parallel Register

CCD Cameras

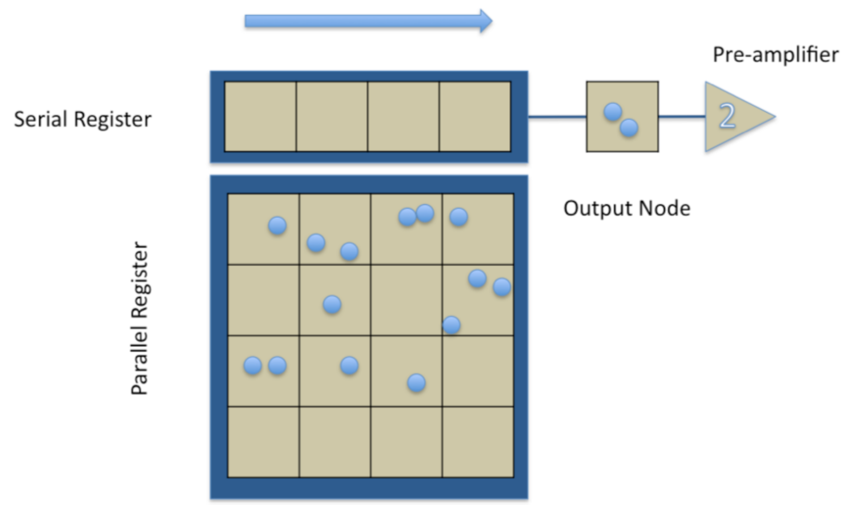


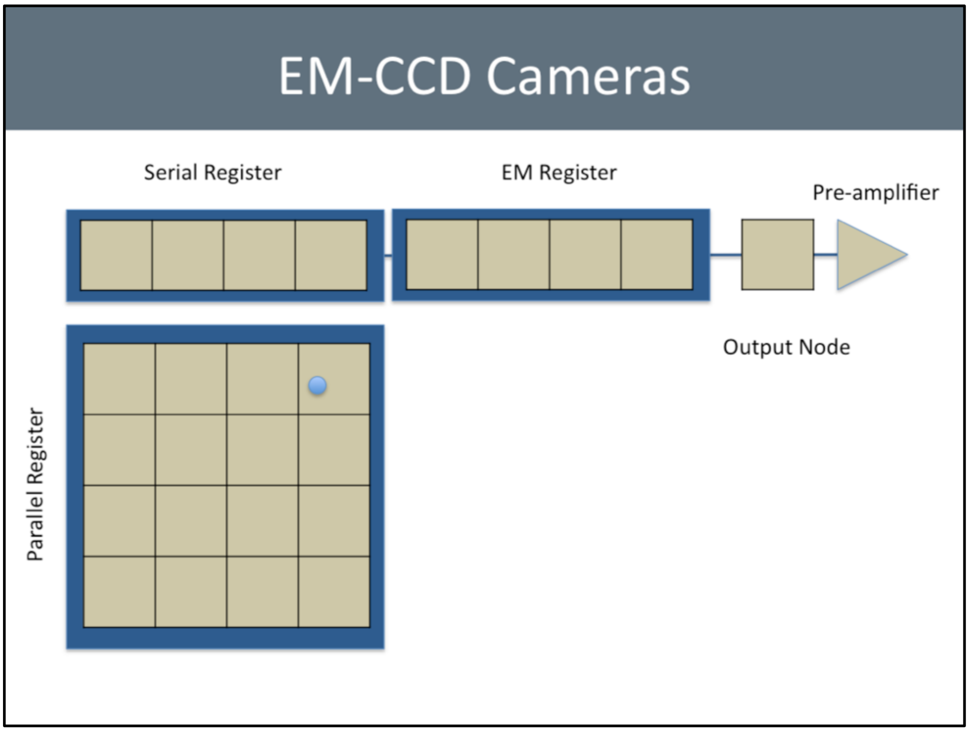
Pre-amplifier
Serial Register
1
Output Node
Parallel Register

CCD Cameras


Pre-amplifier
Serial Register
2
Output Node
Parallel Register

CCD Cameras

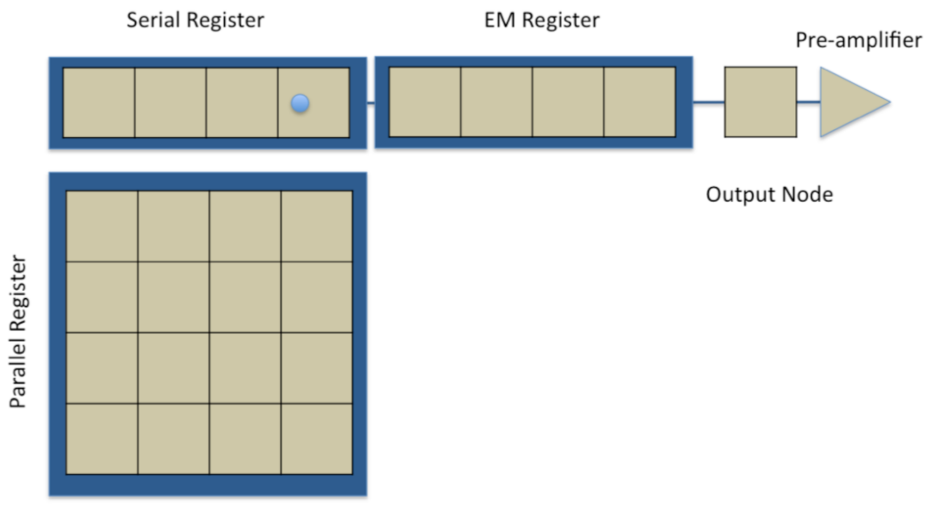

Pre-amplifier
Serial Register
2
Output Node
Parallel Register

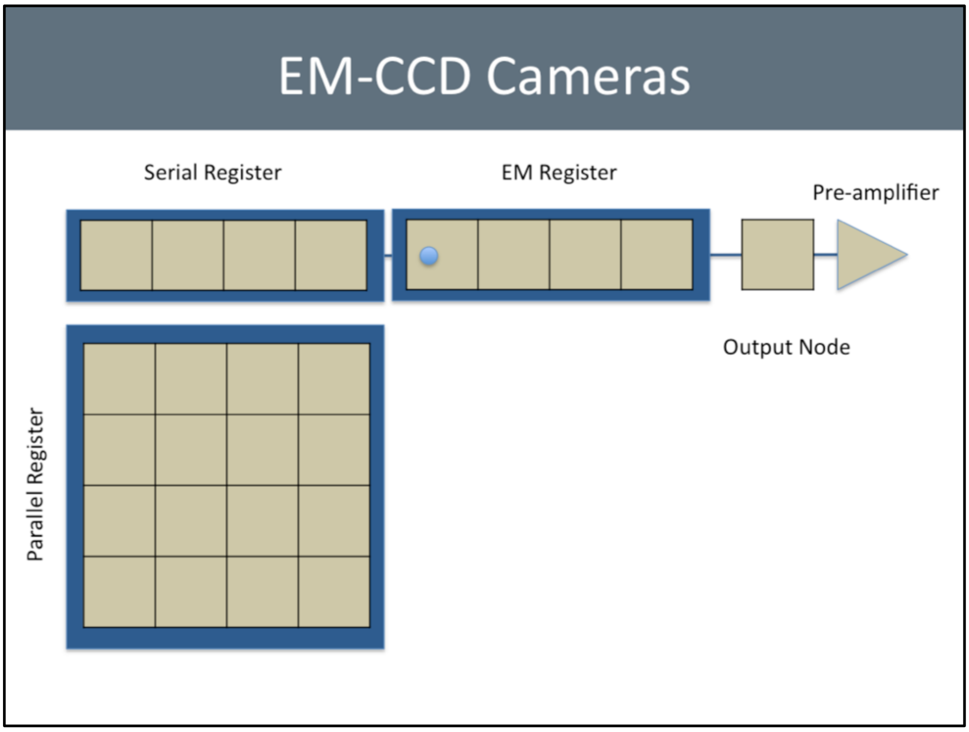


EM-CCD Cameras (Electron-Multiplying)

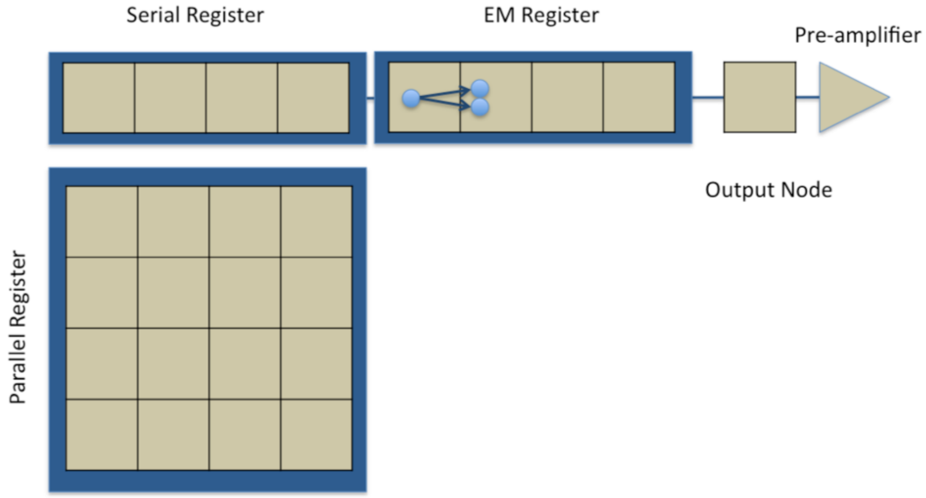
This is just one different flavor of CCD cameras.
These EMCCD cameras contain an extra register – a multiplier which amplifies the signal per photo-reactive cell – these are therefore very useful for imaging specimens at very low light levels.

EM-CCD Cameras

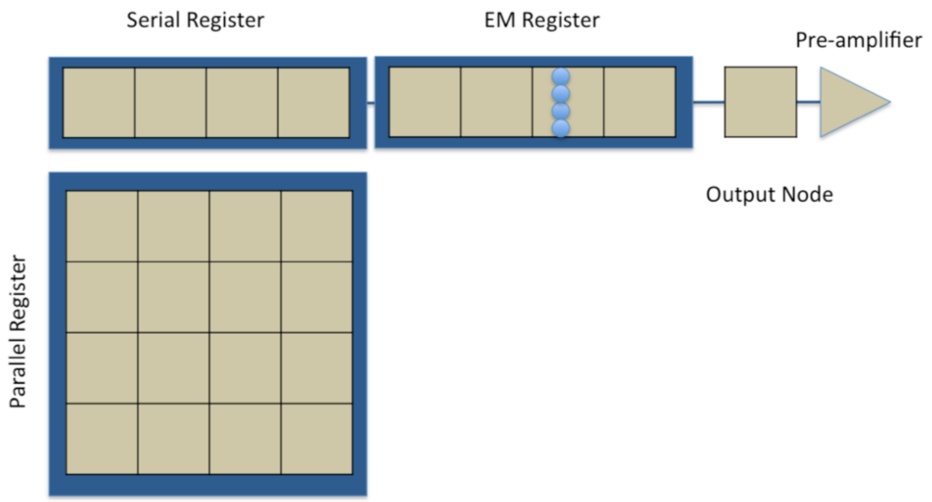



EM-CCD Cameras
Serial Register
EM Register
Pre-amplifier
Output Node
Parallel Register

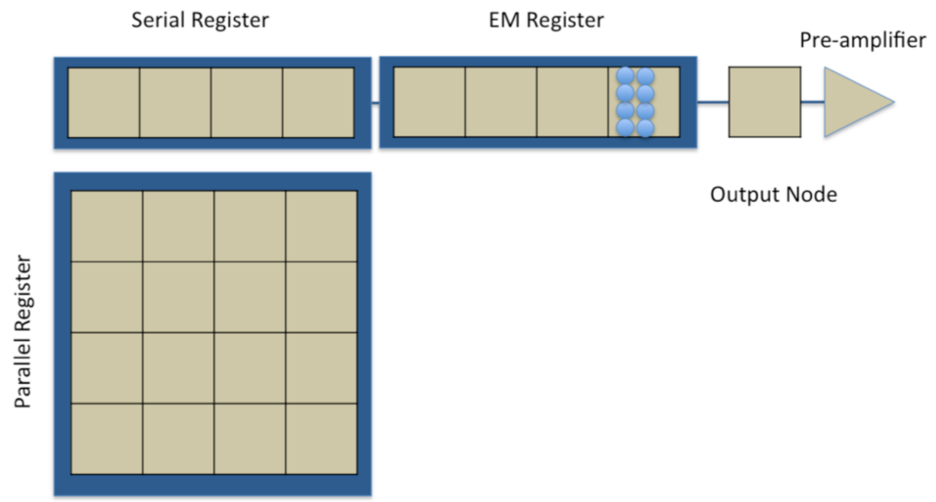
EM-CCD Cameras



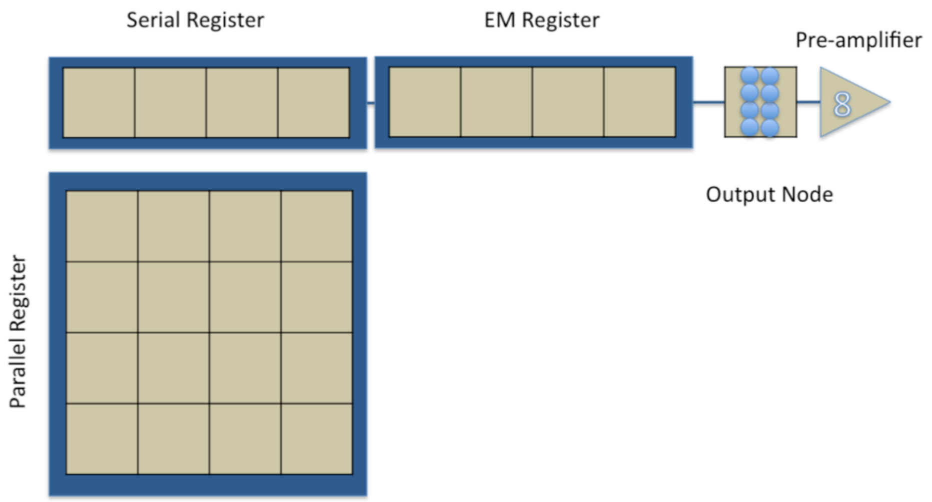
EM-CCD Cameras

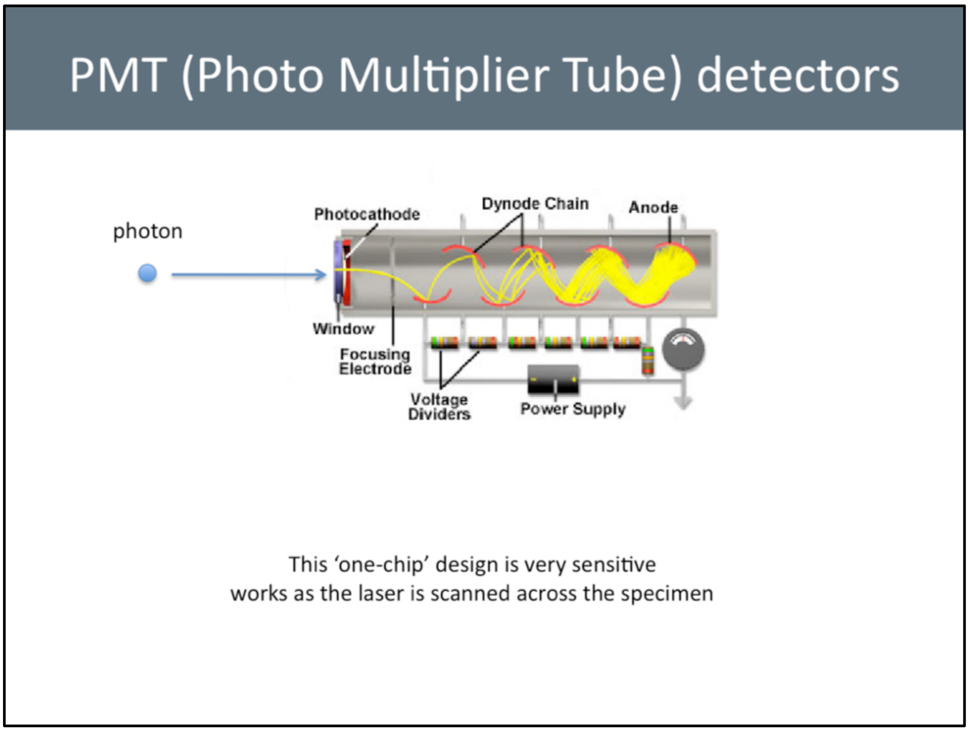


EM-CCD Cameras



EM-CCD Cameras





PMT (Photon Multiplier Tube) Detectors

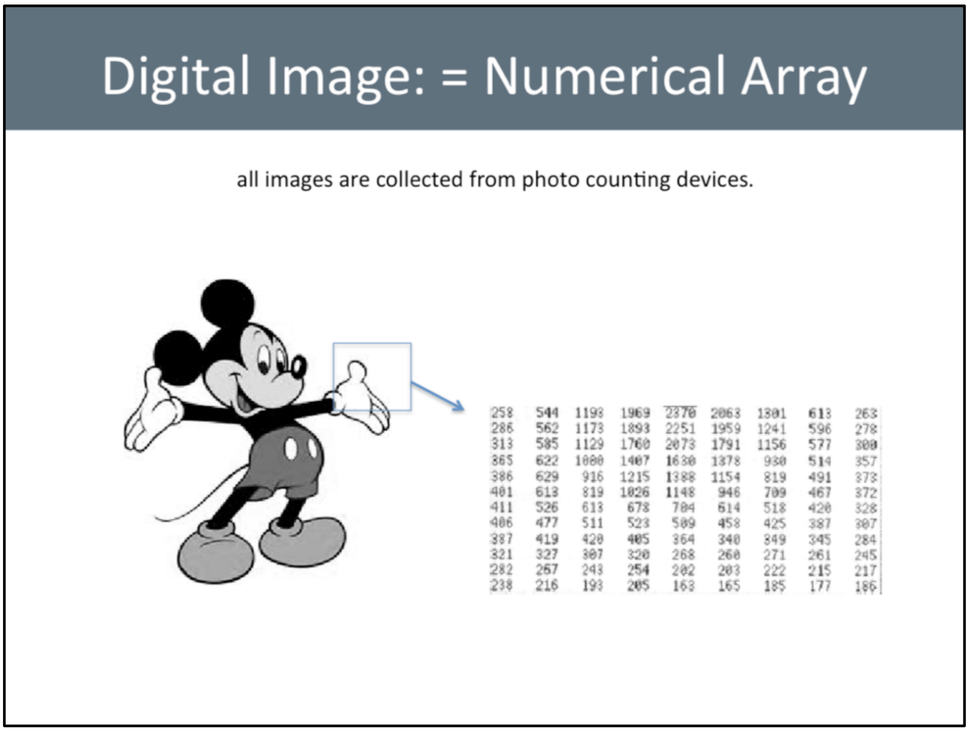
PMT detectors are a second type of photon detector on scanning confocal microscopes. It is important that the image is scanned to generate XY information as PMTs can be thought of as 'one chip' cameras.

PMT detector have a photocathode at the front of the tube which converts one photon hitting the front of the tube into one electron inside the tube.

These are then accelerated through the tube, being amplified as they hit a series of Dynodes.

The extent of amplification depends on the speed the electrons hit the dynodes which is in turn is determined by the voltage difference from the front to the back of the tub.

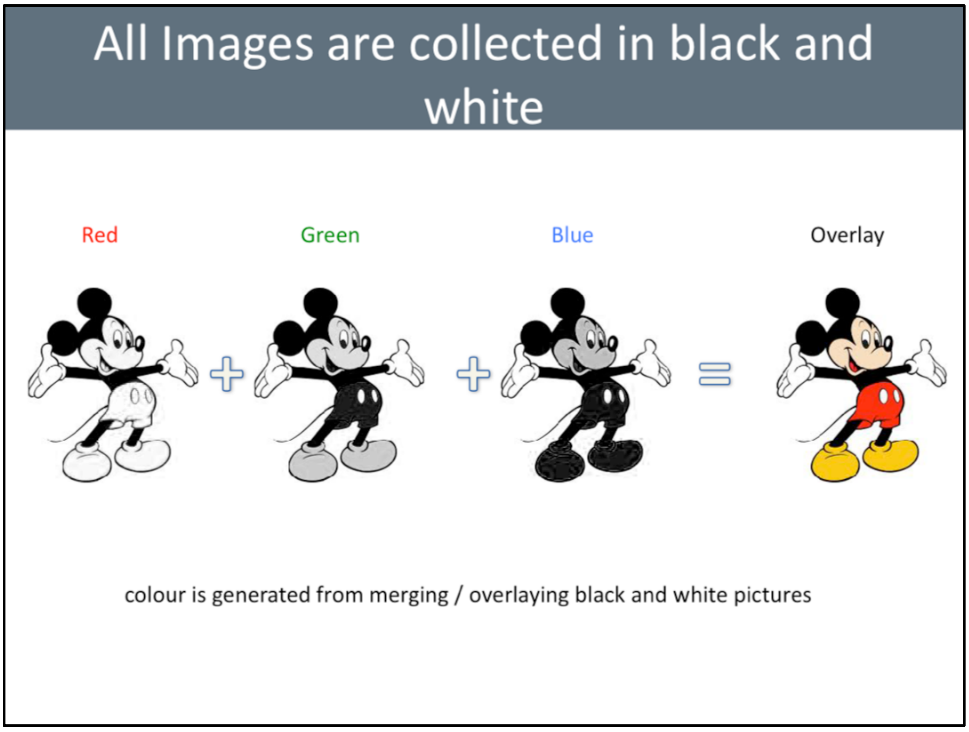
The number of electrons hitting the anode is 'read' and sent to a computer.



Digital Images

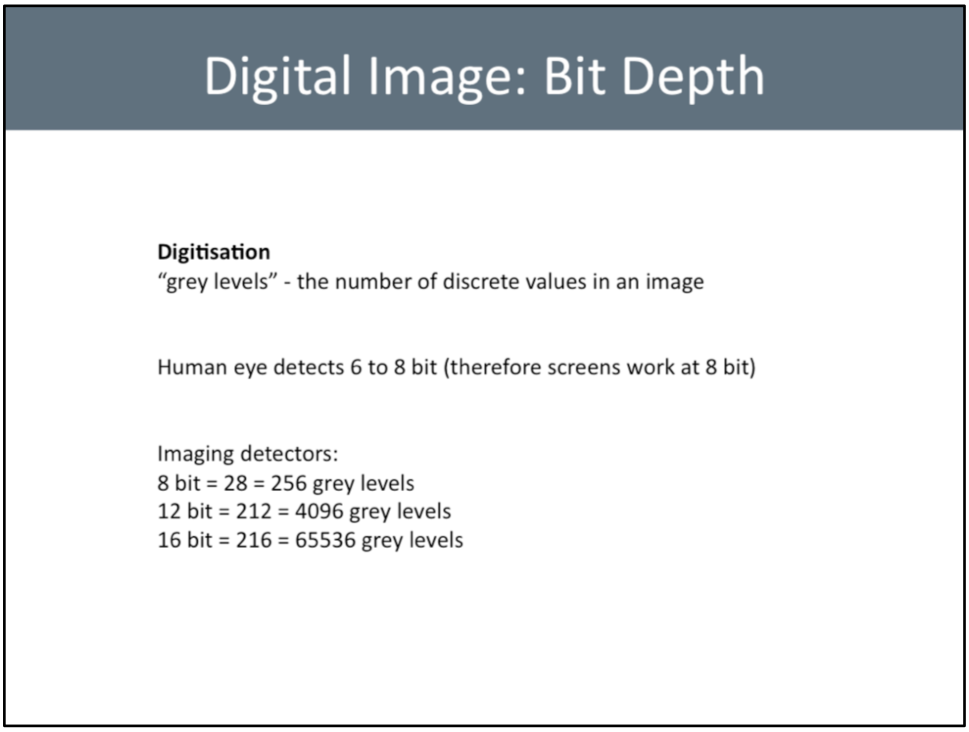
As we have seen, in microscopy all images are collected from photon counting devices.

A consequence of this is that an image is just an array of numbers of photons counted at each pixel.



Digital Images

Another consequence of digital image collection is that all images are just counts of photons and therefore **<u>don't</u>** contain information about color/wavelength. This color information is therefore added subsequently with prior knowledge of the filters and mirrors that the photons have passed through before reaching the camera. Colored images are then just separate black and white images overlayed!



Bit Depth

If we return to our bucket of water analogy for CCD cameras. Imagine that the weatherman measures the depth of water in the bucket using a ruler, and that his ruler has 255 calibrated divisions. He can therefore accurately assess the amount of water in his bucket to one of 256 (255 + zero) values.

This would be described as an 8 bit system (this means there a 2^8 values)

The human eye (and therefore screens) can distinguish between up to roughly 256 different shades of grey.

digital cameras can distinguish between up to 65536 shades of grey (16 bit cameras)

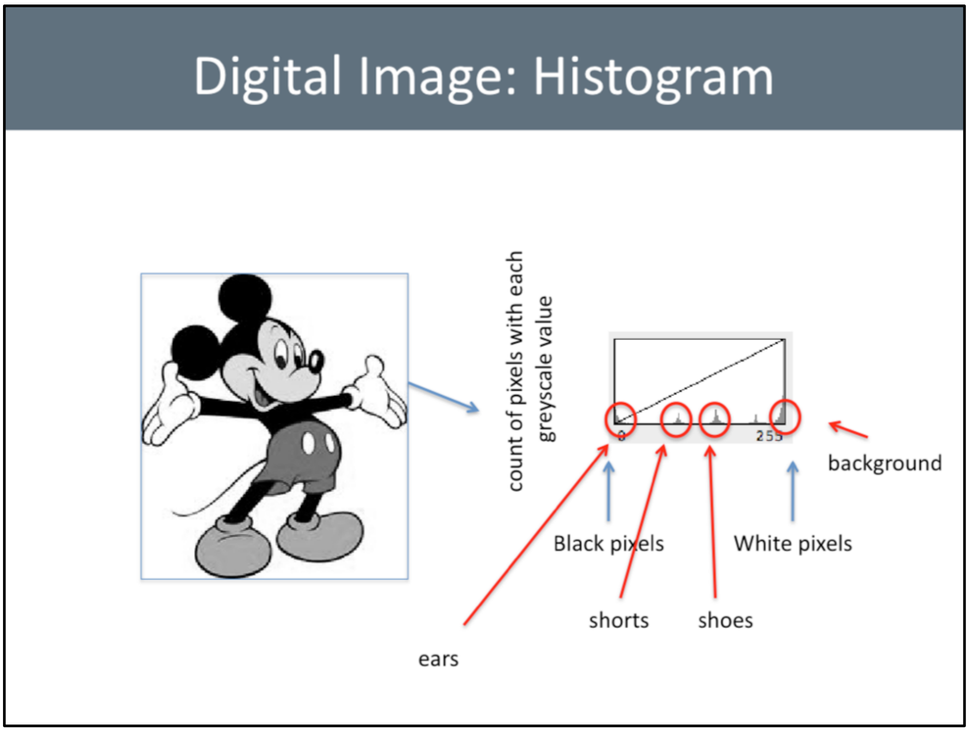
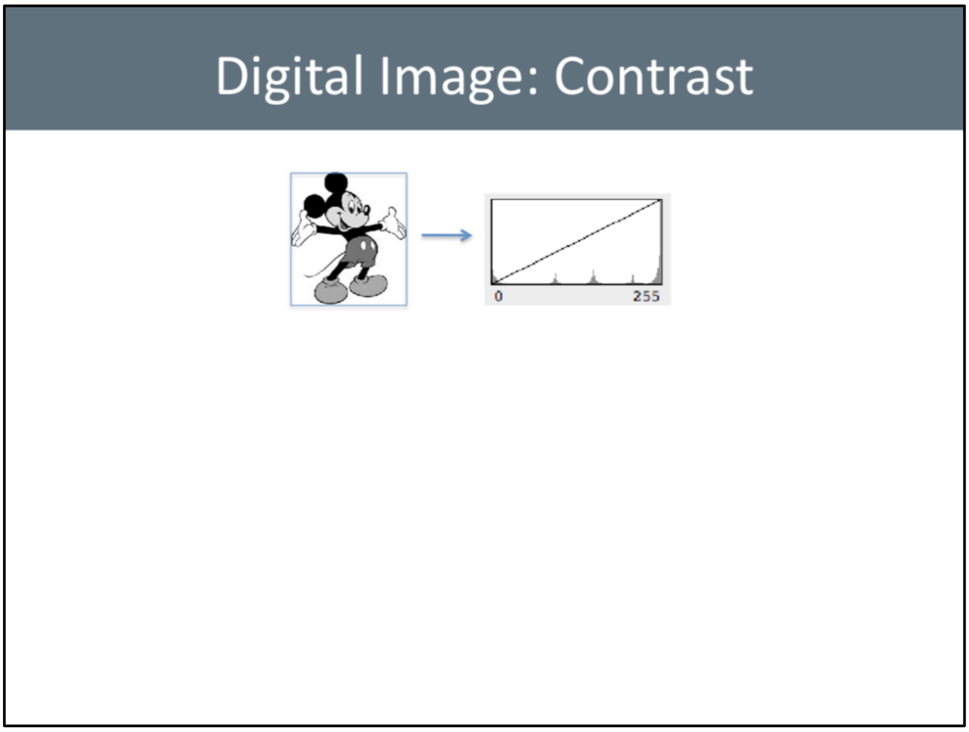


Image Histogram

An image may be represented as a histogram of pixel greyscale values rather than as an image.

In this example an 8 bit Mickey Mouse is shown in histogram form. On the Y axis is the number of pixels in the image with a certain greyscale value, and in the x axis are the different greyscale values (from 0 which are the black pixels – eg Mickey's ear – Through to 255 which are the white pixels of the background.)

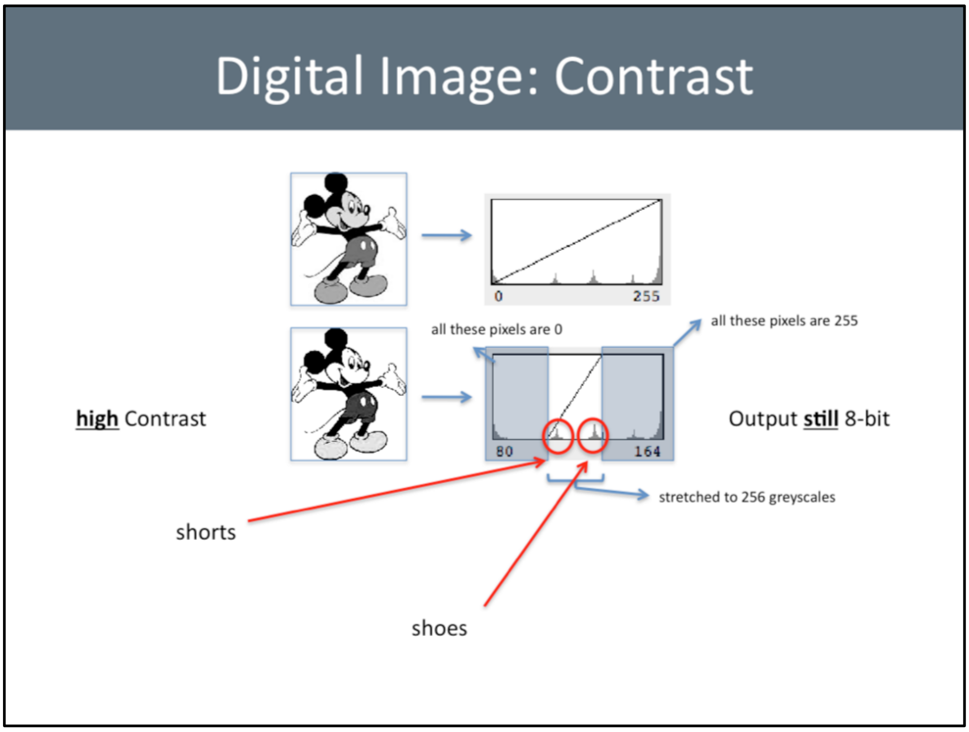
The various peaks of pixel numbers probably represents the greys of Mickeys face, shoes and shorts.



Digital Image: Contrast

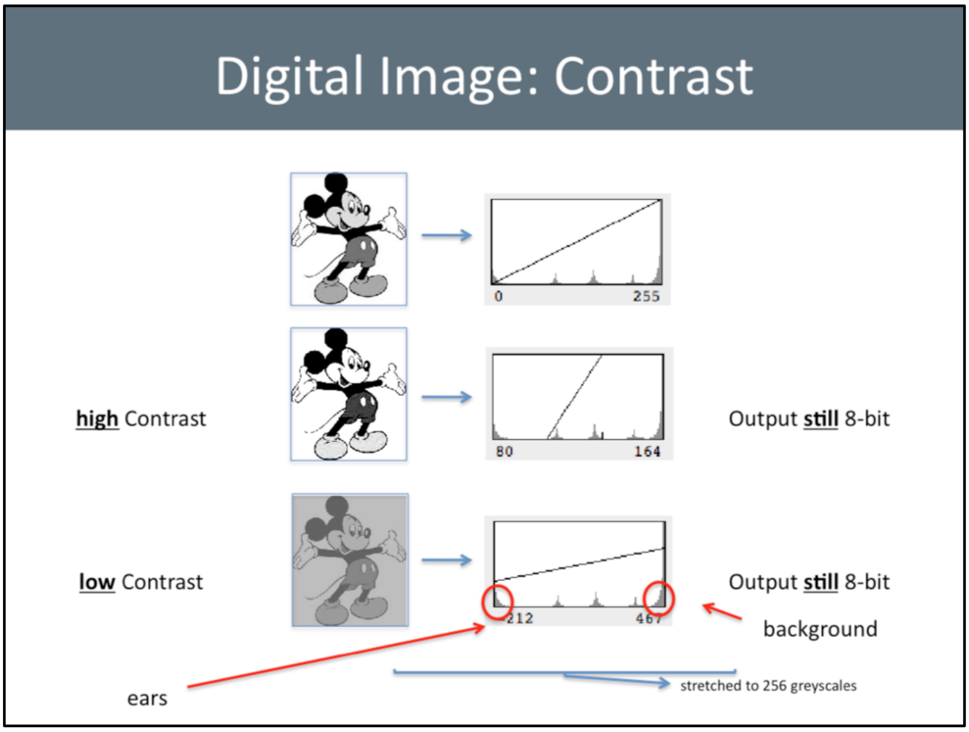
First off we take a look at what changing the contrast in your image means with regards to the image histogram.

This is the same process as changing the contrast on your TV screen.



Digital Image: Contrast

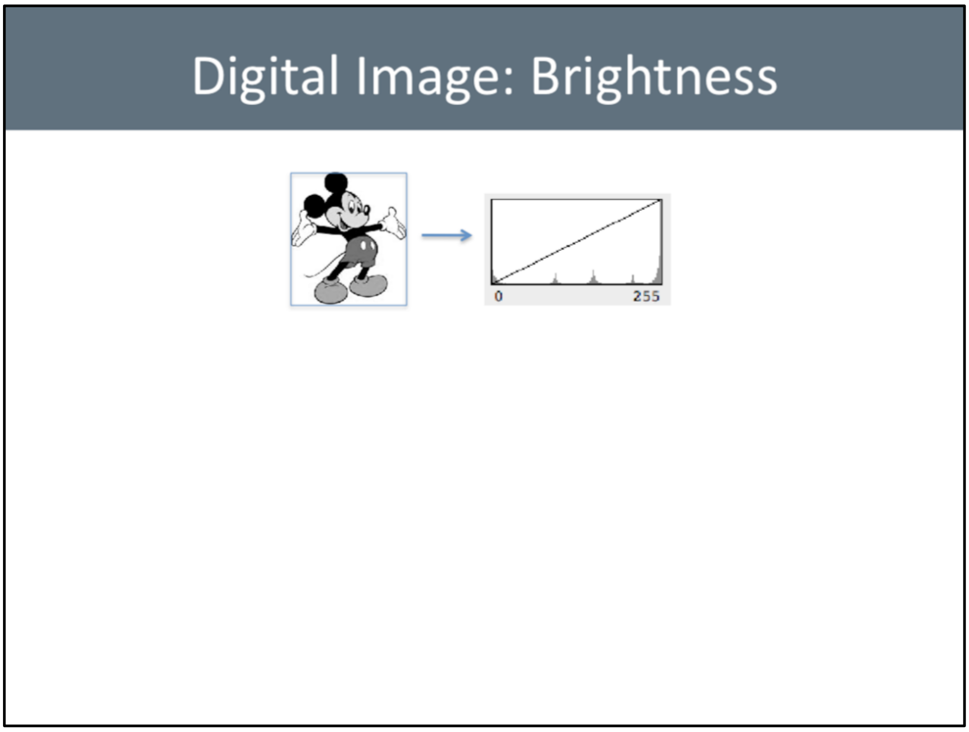
By **increasing** the contrast you are essentially 'cropping' the image histogram and then scaling the smaller X axis to 256 values (the output is always 256 = 8 bit). To the left of the 'cropping' all pixels are given the value zero and are therefore black, and to the right of the 'cropping' all pixels are given the value 255 and are therefore white. In this example the rescaling of the remaining greyscale leads to Mickey's shorts looking darker (value now is smaller) and Mickey's shoes looking lighter (value is now larger).



Digital Image: Contrast

By **decreasing** the contrast you are essentially 'stretching' the image histogram and then scaling the larger X axis to 256 values (the output is always 256 = 8 bit).

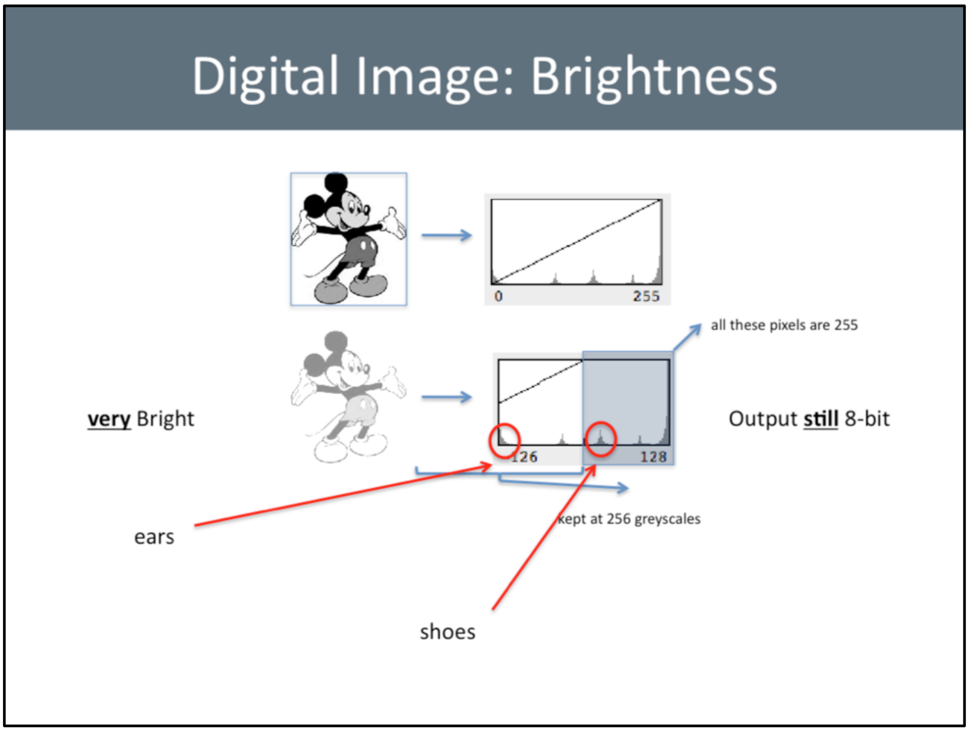
In this example the rescaling of the greyscale values leads to Mickey's ears looking greyer (value now is now half way along the new x axis) and the background looking greyer (value now is now close to the middle of the new x axis).



Digital Image: Brightness

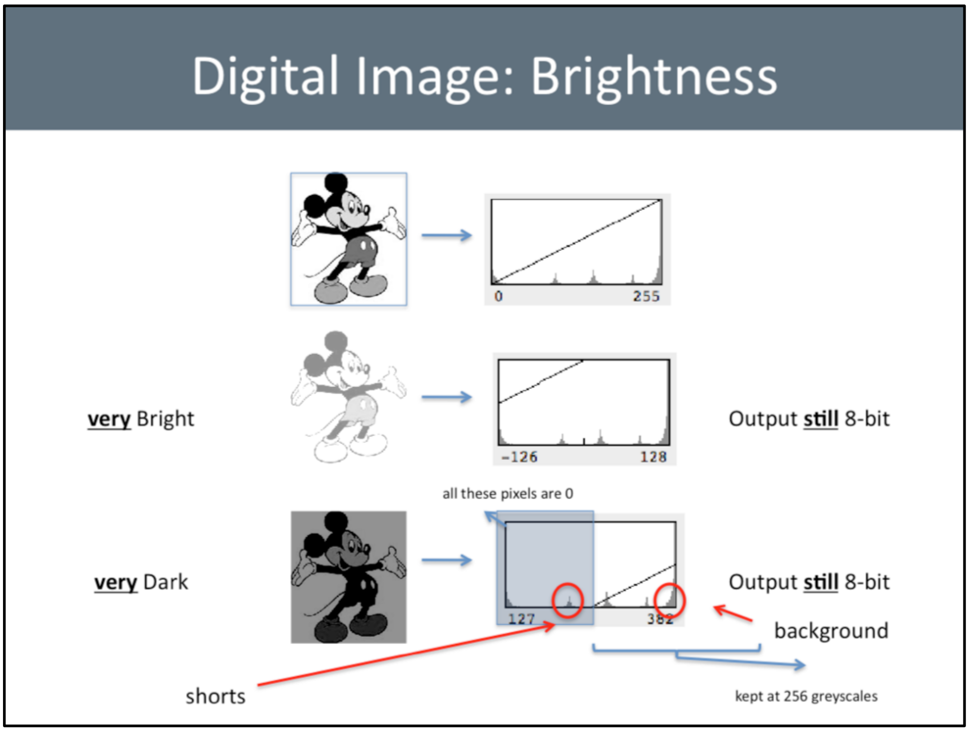
Next e take a look at what changing the brightness in your image means with regards to the image histogram.

This is the same process as changing the brightness on your TV screen.



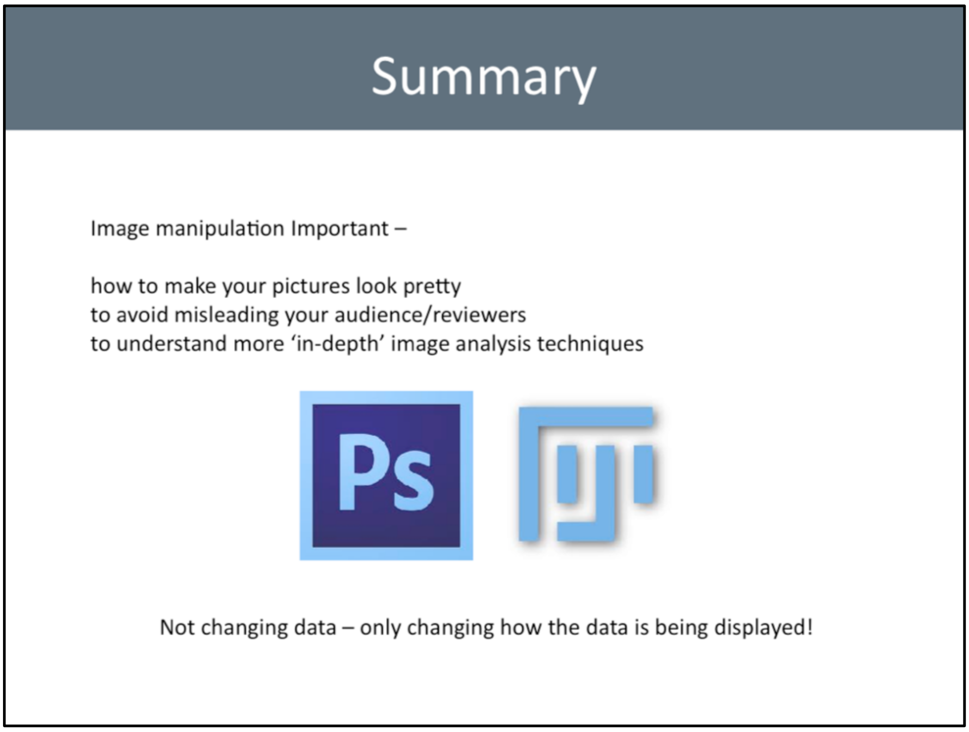
Digital Image: Brightness

By **increasing** the brightness you are essentially 'shifting' the image histogram to the left (the output is always 256 = 8 bit). To the right of the 'shift' all pixels are given the value 255 and are therefore white, and pixels that had the value of zero are now half way along the x and are therefore now grey rather than black. In this example Mickey's shoes now look white (value now 255) and Mickey's ears look greyer (value is now larger).



Digital Image: Brightness

By **decreasing** the brightness you are essentially 'shifting' the image histogram to the right (the output is always 256 = 8 bit). To the left of the 'shift' all pixels are given the value zero and are therefore black, and pixels that had the value of 255 are now half way along the x and are therefore greyer rather than white. In this examples Mickey's shorts now look black (value now 255) and Mickey's ears look grey (value is now smaller).



Important – how to make your pictures look pretty or to how to avoid misleading your audience/reviewers

Not changing data – only changing how the data is being displayed

This kind of image manipulation is routinely carried out using software such as Photoshop (brought) or Image J/FIJI (free).