Biomedical Imaging

& Analysis

Lecture 5. Fall 2014

Image Formation & Visualization (V):

Optical Imaging Systems.

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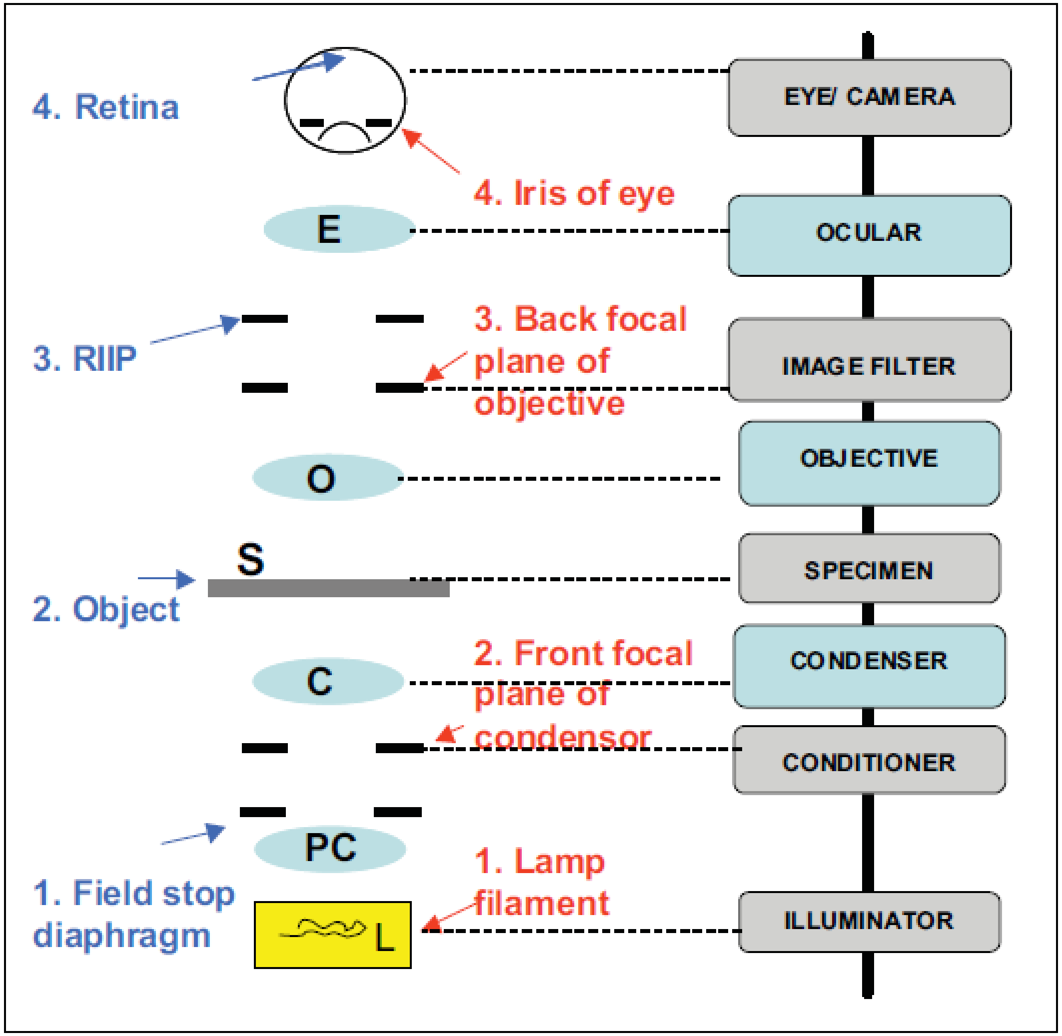
Basic Microscope Setup



- How a Lens Works
- Diffraction Pattern
- Linear System's view of an optical system

From text: Microscopic Image Analysis for Life Science Applications

Performance Metrics of a Microscope

- Resolution: the smallest distance that can be resolved.

- Field of view: the area of a specimen that can be observed and recorded in an image.

- Depth-of-field: the axial distance (depth) in the specimen that appears in focus in an image.

- Light collection power: it determines image brightness.
  – Image bit depth: A 16 bit images have a finer discretization of the range of intensity data stored than an 8 bit image, at each pixel / voxel. Without recording ability the light collection power of a microscope (or telescope for that matter) cannot be taken advantage of…

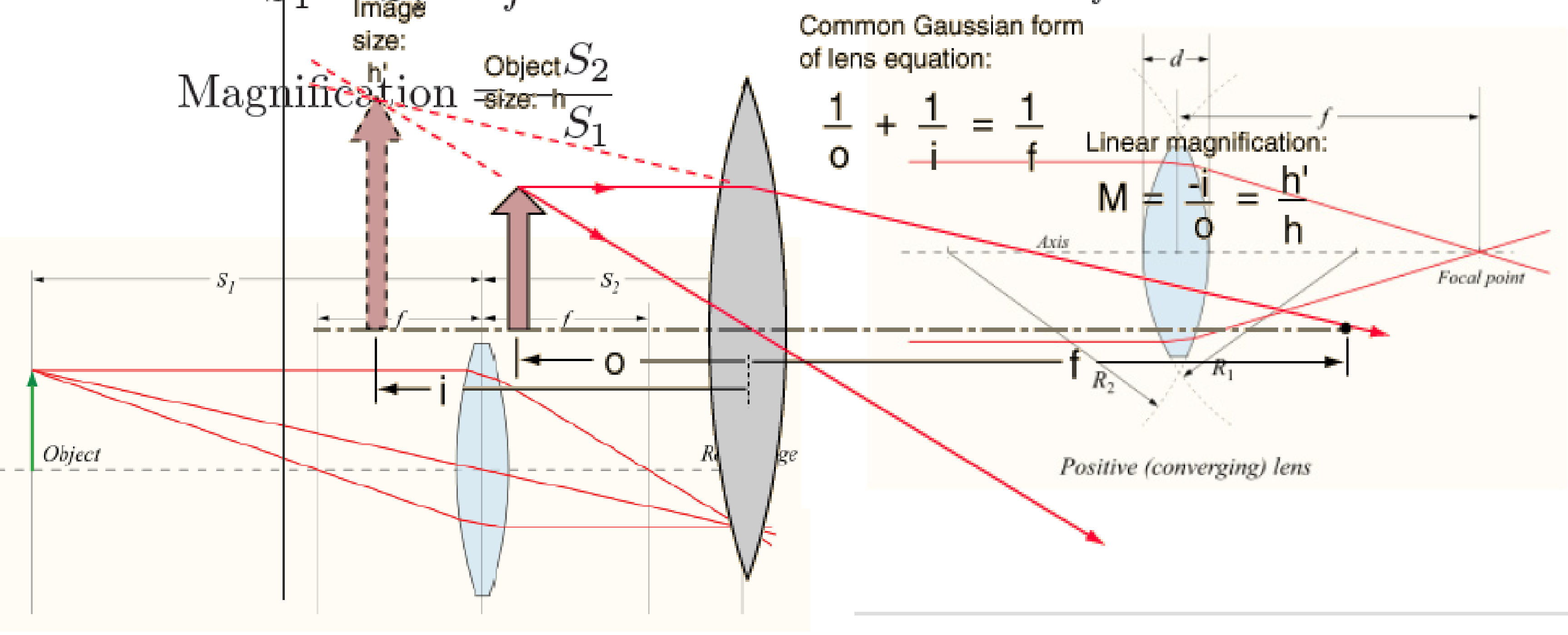
Optics: Some Basics (I)

- Lens and Magnification (courtesy, Wikipedia)
- Thin lens approximation (a simple math model)
- Real v/s Imaginary Images

$$\frac{1}{S_1} + \frac{1}{S_2} = \frac{1}{f}$$

Focal point f: $\frac{1}{f} = (n-1)\left(1/R_1 + 1/R_2\right)$

$$\text{Magnification} = \frac{S_2}{S_1}$$



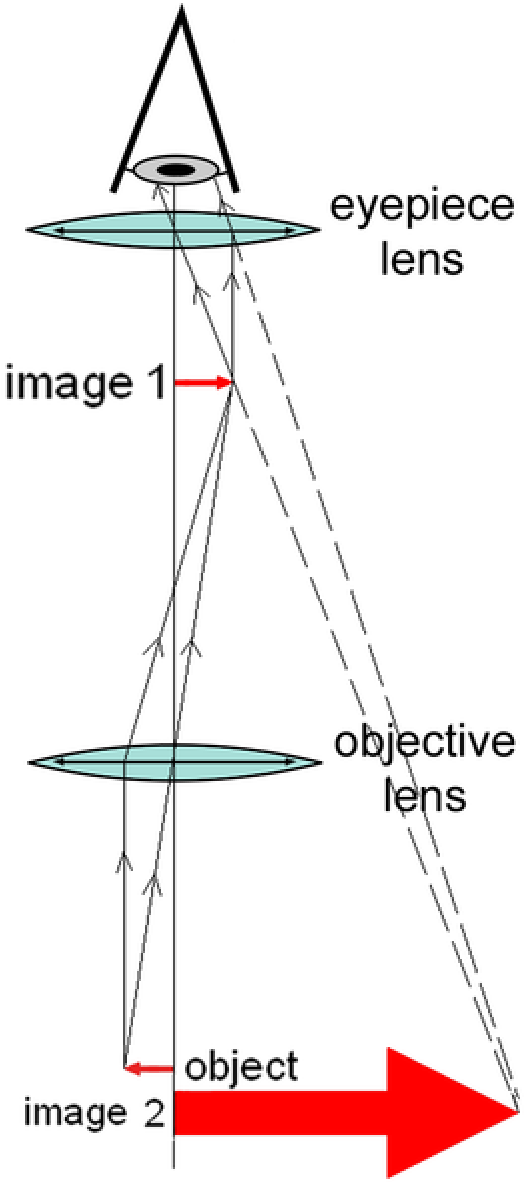
Lenses of the Compound Microscope

Gives the viewer an erect enlarged virtual image.

Oculars (eyepieces): provide some magnification, focus image at eye (can be 1 or 2 of them)

Objectives: located on rotating nosepiece, provide magnification and resolving power (may be 3 to 6 of them)

Condenser: located under stage, focuses light on the   specimen. It may also contain an iris diaphragm that controls the size of the cone of light entering the condenser.



Magnification

The overall magnification is given as the product of the lenses and the distance over which the image is projected:

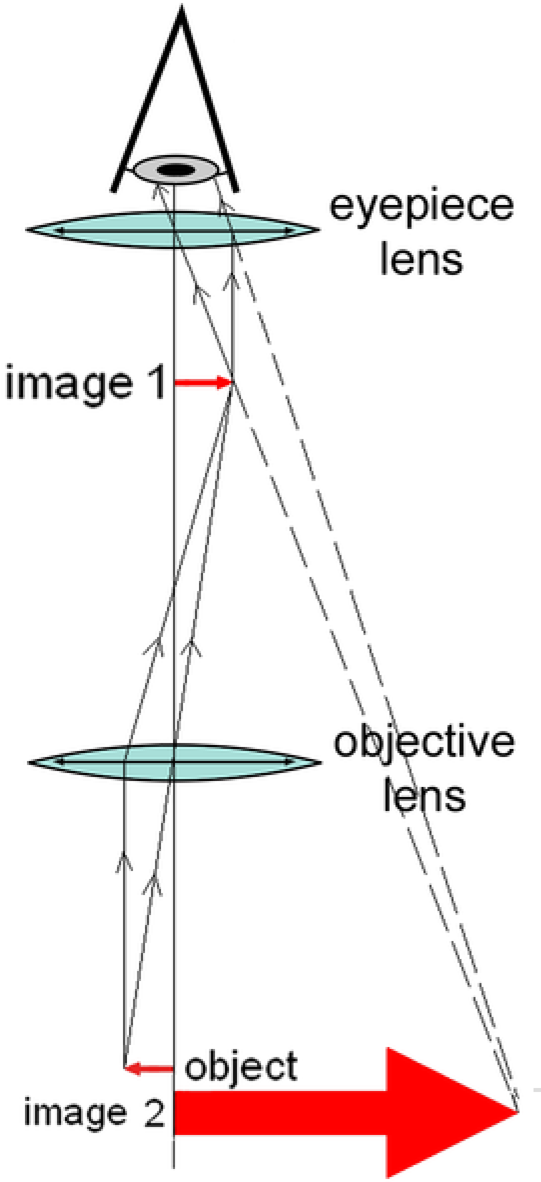
$$M = \frac{D \cdot M_1 \cdot M_2}{250mm}$$

where:

D = projection (tube) length (usually = 250 mm);

M_1, M_2 = magnification of objective and ocular.

250 mm = minimum distance of distinct vision for 20/20 eyes.



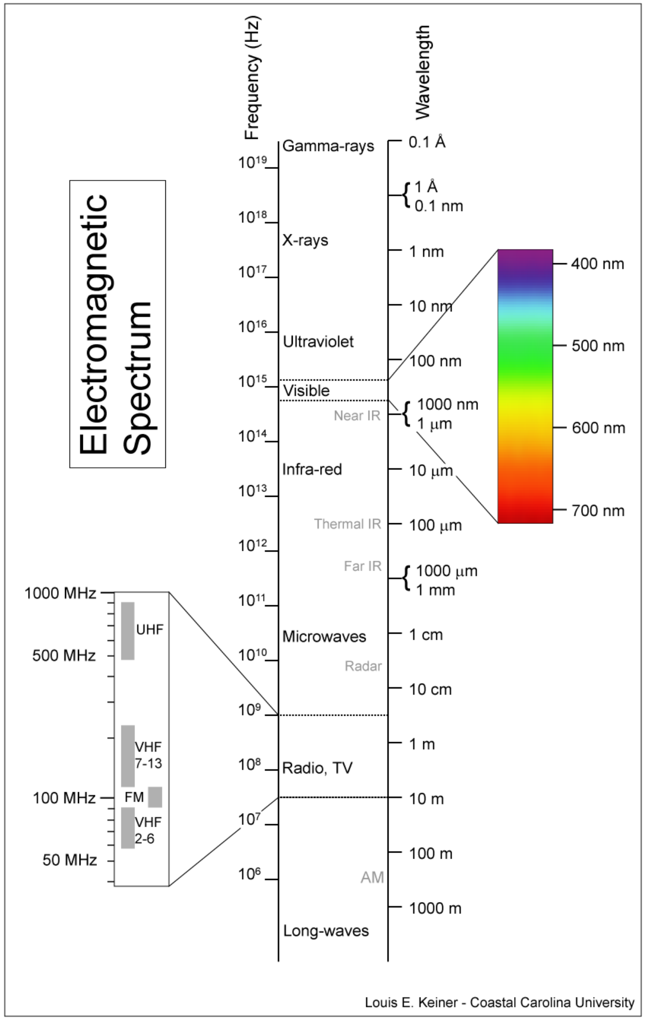
Photon Energy (II)

- Energy of a photon: Planck's law

$$E = h\nu = h\frac{c}{\lambda}$$

h: Planck's constant; 6.626×10^{-34} J·s
ν: frequency of light;
λ: wavelength of light
c: speed of light

energy of a photon =
3.973×10^{-19} J at 500nm



- Shorter waves have higher energy.

Microscope Modeling – breaking optical imaging down to a science…

What is a Linear System..? **A*x*** = ***b***

Linear transforms you probably know :

- Rotation, translation, scaling – spatial transforms
- Convolution

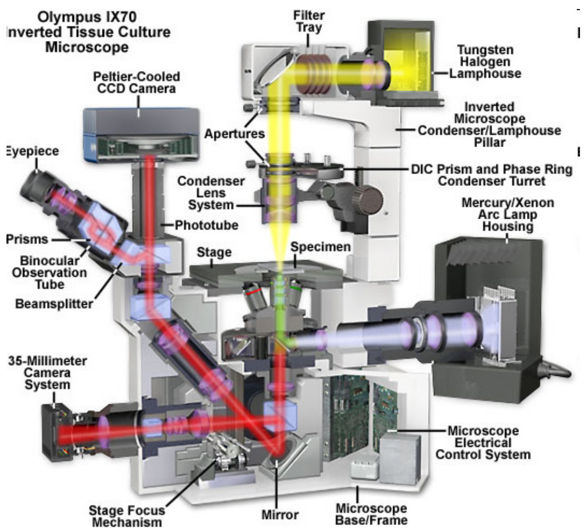
Wolfram – definition of a linear operator:

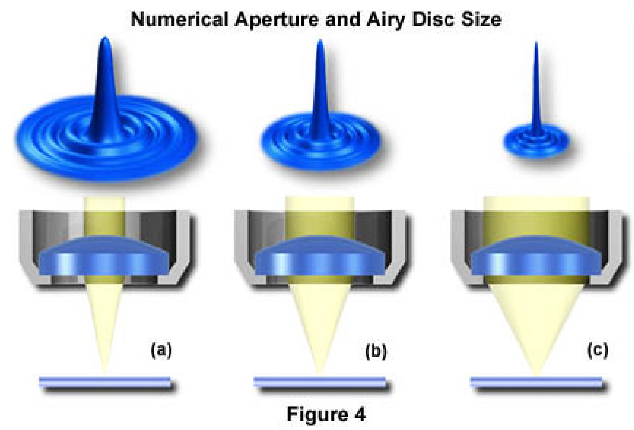
A linear transformation between two vector spaces V and W is a map $T : V \to W$ such that

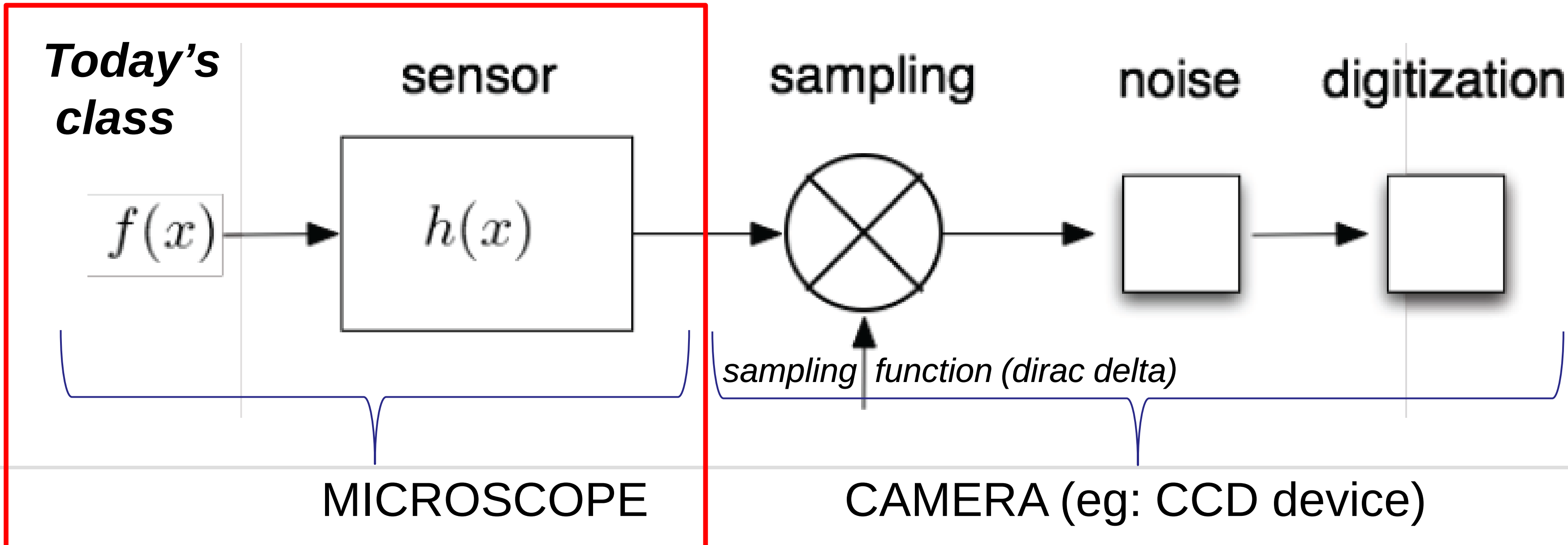
1. $T(\mathbf{v}_1 + \mathbf{v}_2) = T(\mathbf{v}_1) + T(\mathbf{v}_2)$ for any vectors $\mathbf{v}_1$ and $\mathbf{v}_2$ in V, and

2. $T(\alpha\,\mathbf{v}) = \alpha\,T(\mathbf{v})$ for any scalar α.

A Microscope can be modeled as a Linear System







Review: Basic Concepts

- An image records spatiotemporal information of a biological process.

- An image can be considered as both a matrix and a surface.

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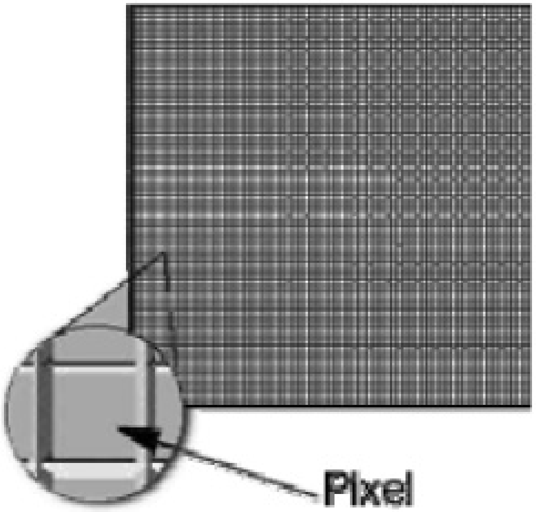
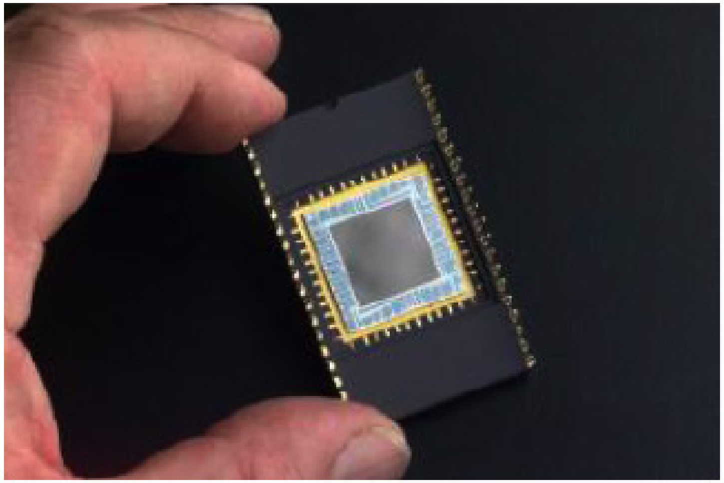
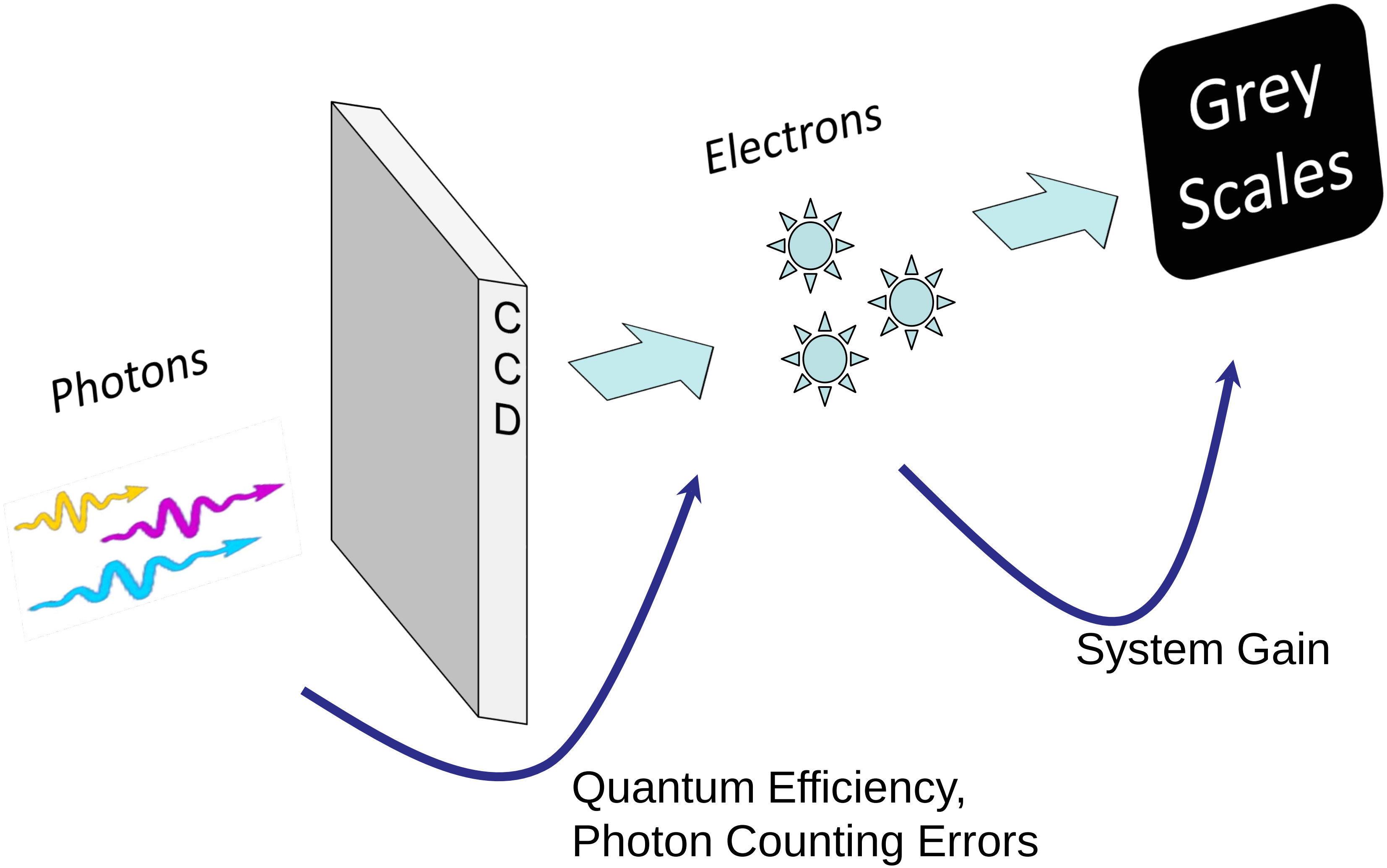
N_{dar}

N_{can}





Recording the Image: what is actually happening at each Pixel?



Camera Noise Model

- Signal $S = I \cdot QE \cdot T$

  T: exposure/integration time
  QE: quantum efficiency

- Signal shot noise

$$N_{shot} = \sqrt{S}$$

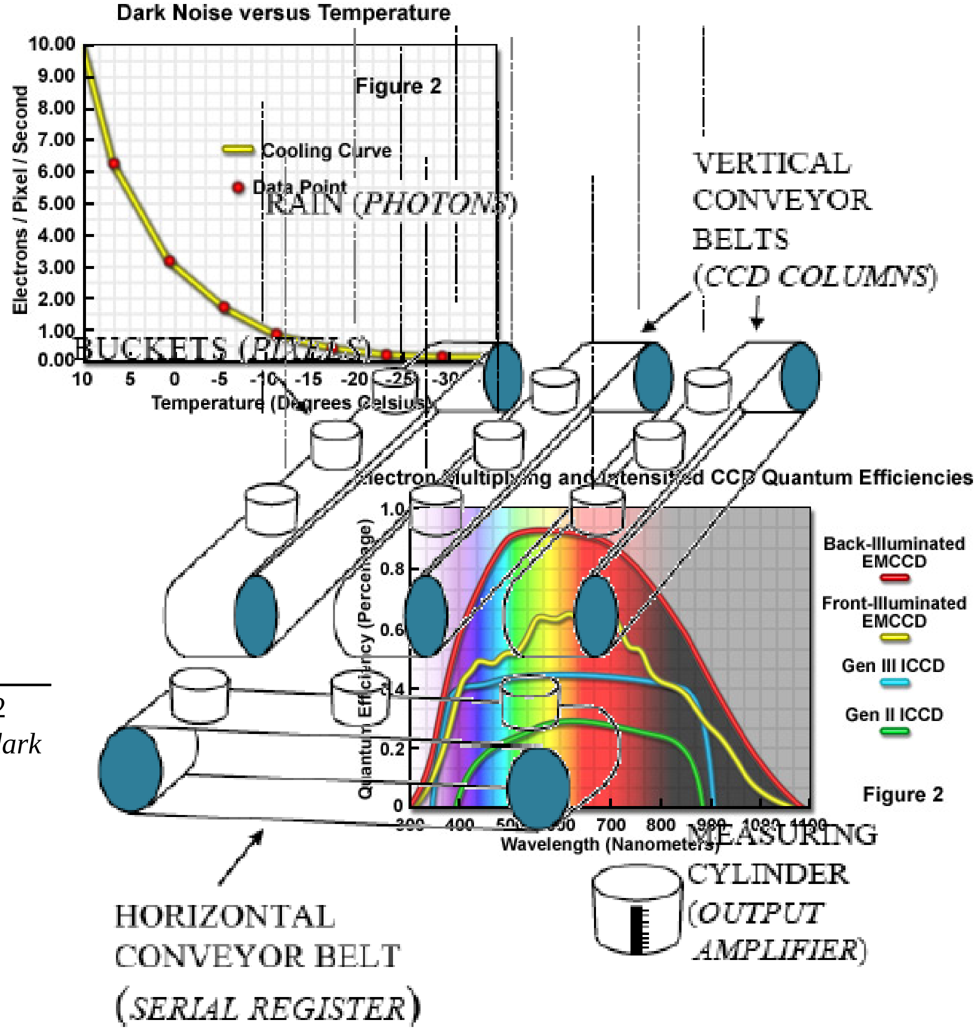
- Camera noise

$$N_{dark} = \sqrt{D \cdot T} \qquad N_{camera} = \sqrt{N_{read}^2 + N_{dark}^2}$$

  D: dark current

- Total noise

$$N_{total} = \sqrt{N_{shot}^2 + N_{read}^2 + N_{dark}^2}$$



Read Noise

- Minimized by careful electronic design

- Under low-light/low-signal conditions where read noise exceeds photon noise, data is read noise limited

- Read noise is not as significant in high-signal applications

- Read noise = std* system gain* 0.707

  (std of subtracted bias images)

Reading all the buckets - what's my Error?

Dark Current

- Dark Current is created by heat and cosmic noise and can be reduced by cooling
- Dark Current builds over time unlike read noise
- Dark current reduction is sensor dependent
- For example, some sensors will halve dark current for every 7 degrees of cooling; some require more cooling
- Other technologies can be applied which reduce the cooling required

Retiga SRV (cooled to -30) Dark Current 0.15 e/p/s
Exi Blue (cooled to zero) Dark Current 0.005 e/p/s