6581/ 20.482

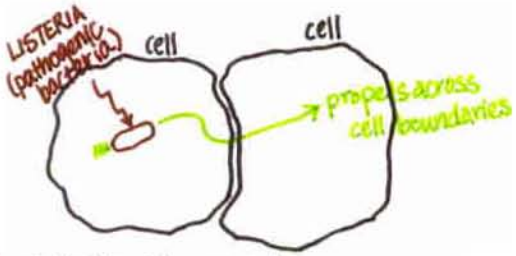
TUESDAY
25 APRIL 2006

LECTURE 19: 2-D & 3-D LIGHT MICROSCOPY; IMAGE RECONSTRUCTION

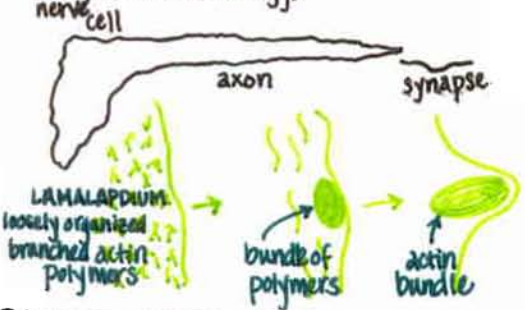
WHAT TYPES OF PHENOMENA MIGHT ONE STUDY?

MOVIES – Time dependent

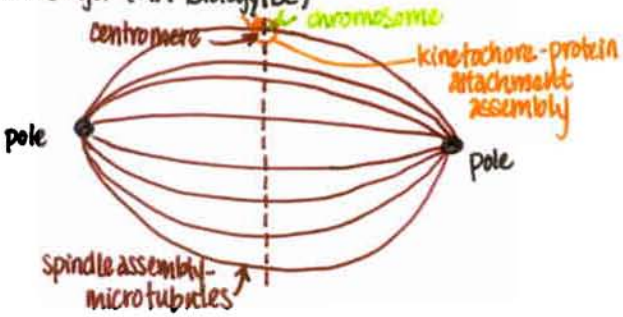
(1) Julie Theriot (Stanford) – Invasive Bacteria



(2) Frank Gertler (MIT Biology)

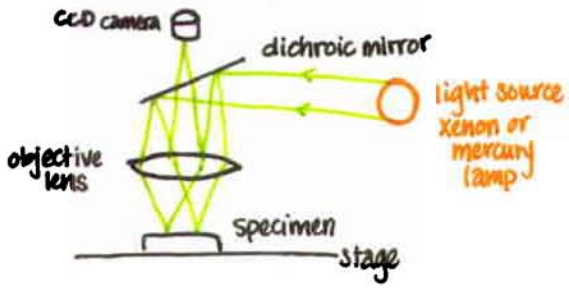


(3) Pete Sorger (MIT Biology/BE)

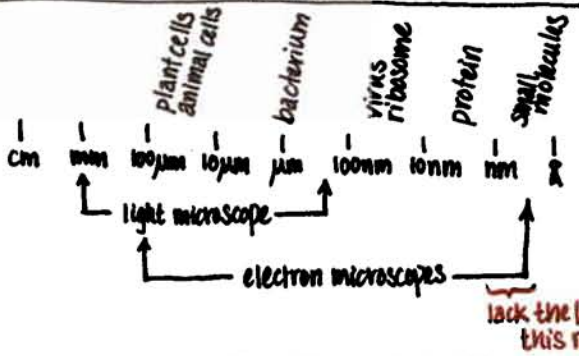


THE PHYSICAL MICROSCOPE – FLUORESCENCE

(1) Wide-field microscope



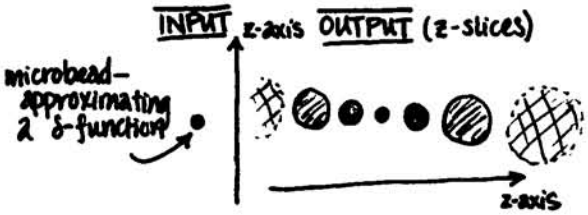
(2) Scanning Confocal Microscope



Resolution Limit: How close can two points be & still be resolved?

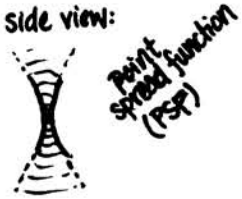
$$D = \frac{0.61\lambda}{N\sin\alpha} \sim 0.2\,\mu m \cong 200\,nm = 2{,}000\,\text{Å}$$

(λ: wavelength of light; N: index of refraction (1.5 for oil); Bragg criteria)



WHY?

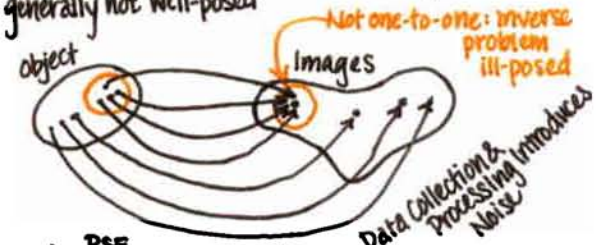
1) Objective lens & aperature don't collect "all" the light
2) Diffraction off edge of aperature (ring pattern)



THE BASIC PROBLEM:

input object → PSF → output image

Mathematical procedure for (L ⟶ R) is well defined. However, we need to solve the inverse problem which is generally not well-posed



Object → PSF → Noise-Free Image → Actual Image

Noise-free image is a convolution of the object with a PSF

$$\text{noise-free image: } g^*(\vec{x}) = \int K(\vec{x},\vec{x}')\, f^*(\vec{x}')\, d\vec{x}' \quad (K: \text{PSF};\ f^*: \text{object})$$

if $f^*(\vec{x}') = \delta(\vec{x}'-\vec{x}_0)$ $\Rightarrow g^*(\vec{x}) = K(\vec{x},\vec{x}_0)$

Actual image

$$g(\vec{x}) = g^*(\vec{x}) + w(\vec{x}) = \int K(\vec{x},\vec{x}')\, f^*(\vec{x}')\, d\vec{x}' + w(\vec{x})$$

Often we treat the PSF as positionally invariant: $K(\vec{x},\vec{x}') = K(\vec{x}-\vec{x}')$