Watershed, Biosensors

- Last class
  - SIM
  - Fancy SIM
- This class
  - Biosensors
  - Schemes
  - Practical thoughts

Watershed

- Common means to segment cells
- Segmenting is hard, watershed may help

Flow of image processing

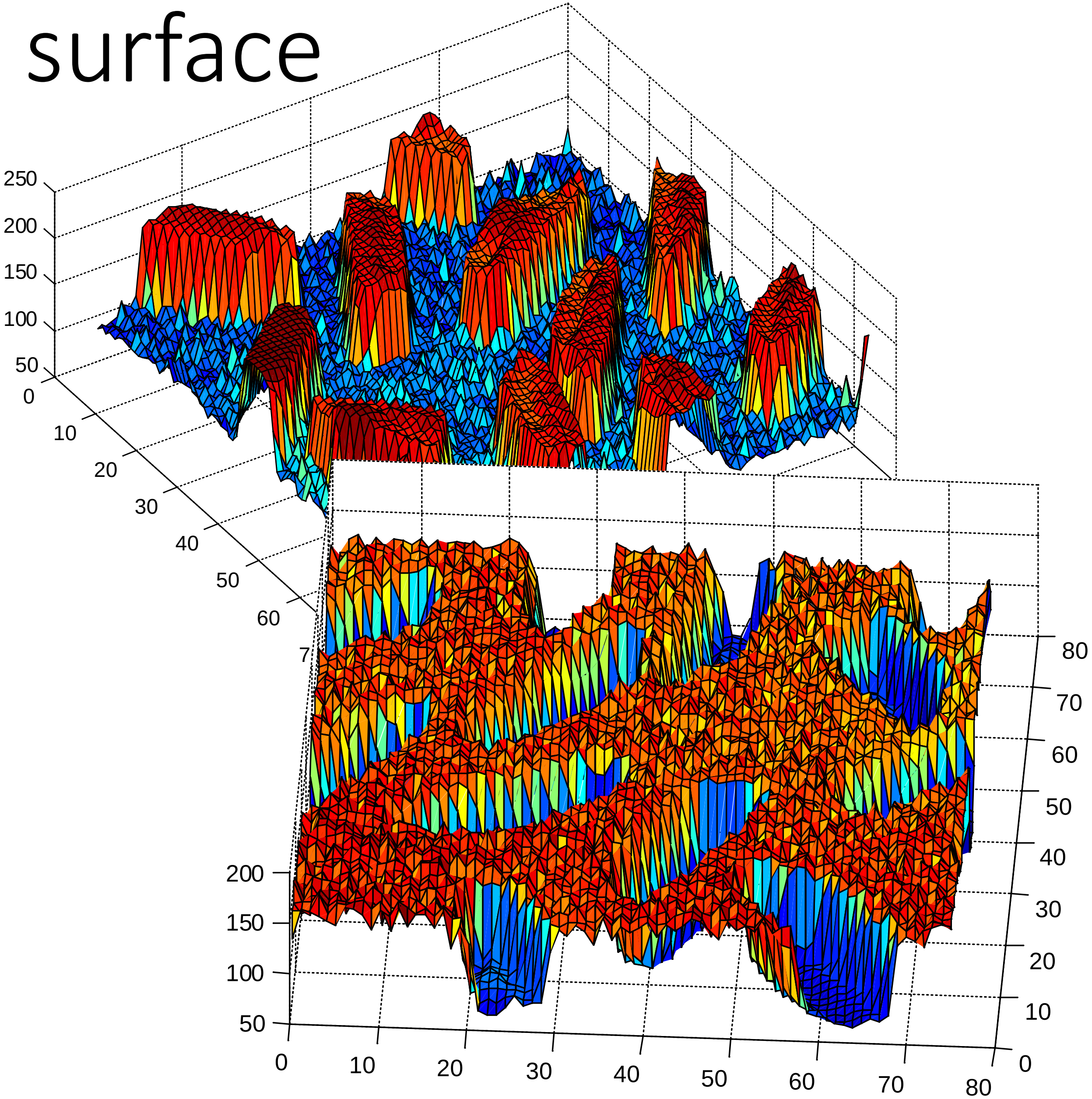
1. Preprocess image
    1. Reduce noise
    2. Even illumination
2. Segment features
    1. Identify individual cells/tissues, etc…
    2. Separate them from background
3. Extract features
    1. Pull out image characteristics
    2. Pull data from least processed image possible

Morphological operators
Geometric operators
2D Fourier transforms

Global thresholds

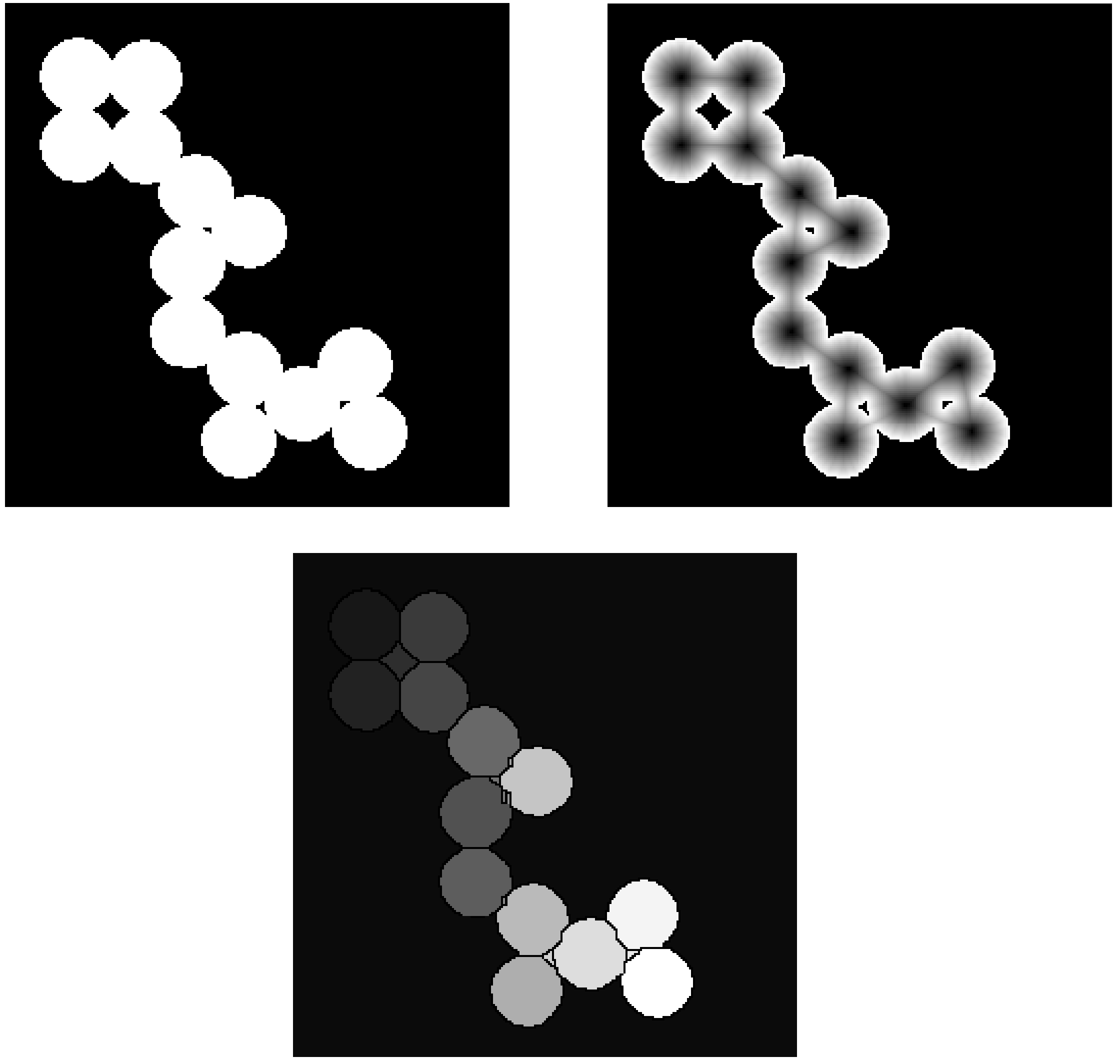
Regionprops

Think of intensity as surface



Watershed fills

- Your goal is to get each object as a basin
- Watershed will fill in until it reaches the background or comes into contact with another watershed
- If you've marked all your features, they will all be marked

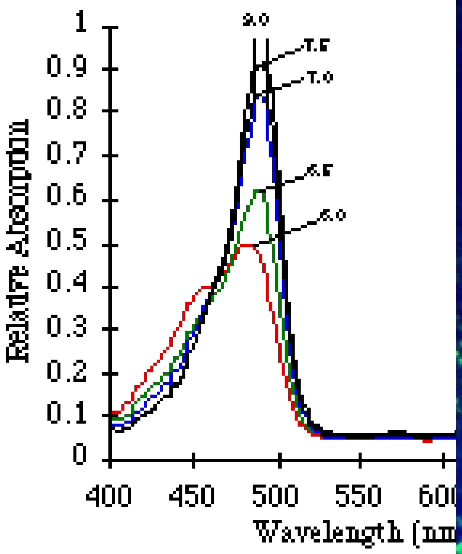


Biosensors

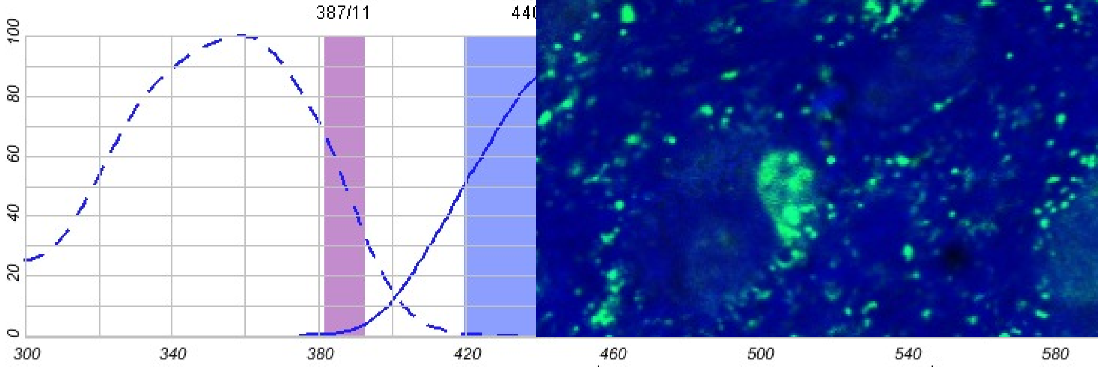
- Using fluorescence to learn about cellular function

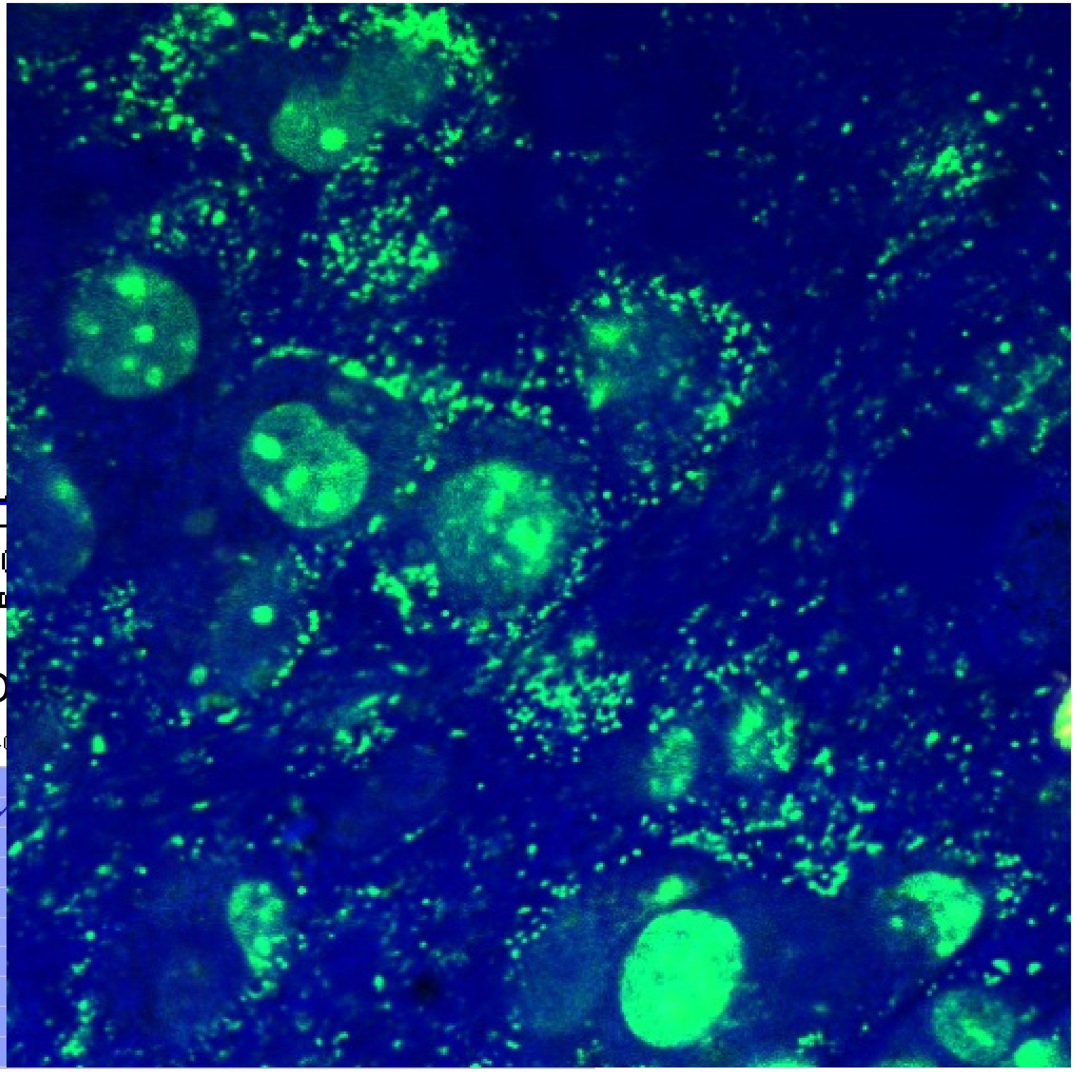
Biosensor = change in fluorescence depending on environment

- If we change the # fluorophores, quantum yield or absorbance upon changes in environment, that is now a sensor
- Fluorescein – pH and hydrophobicity sensor
- DAPI – DNA sensor



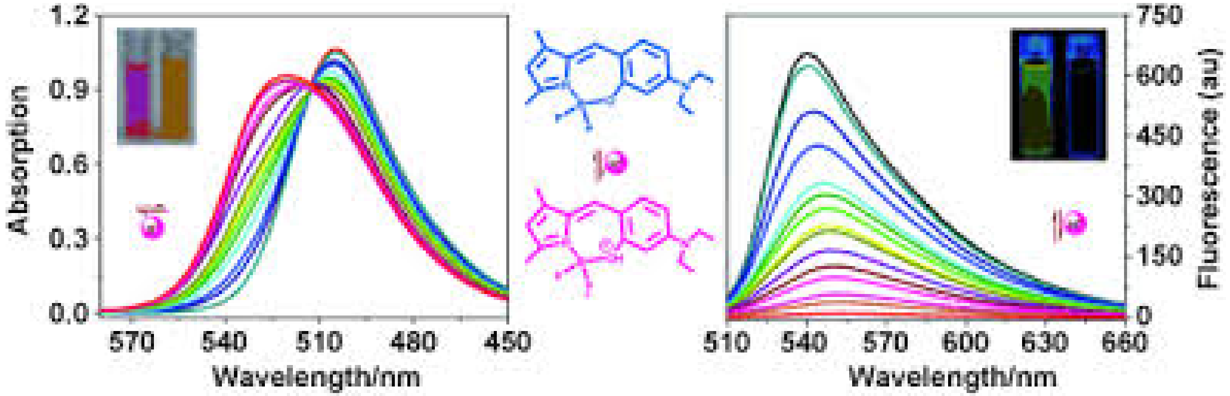
Bound D

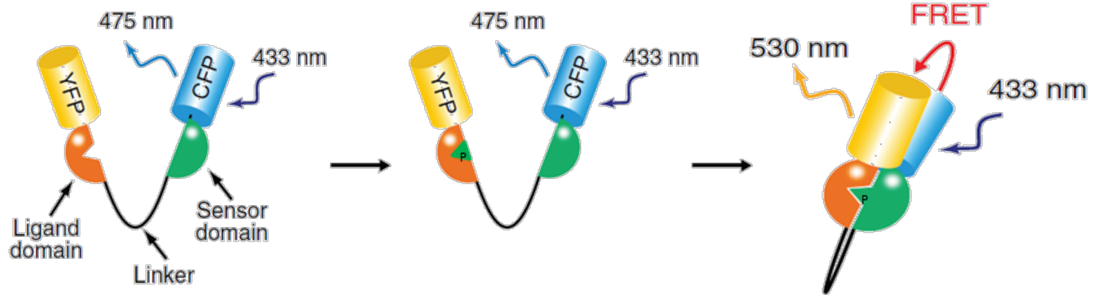


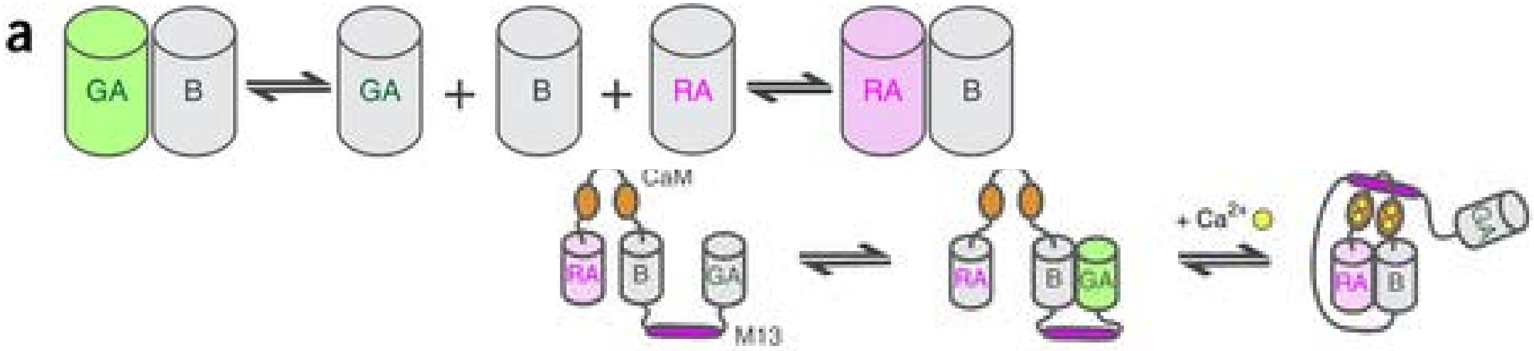


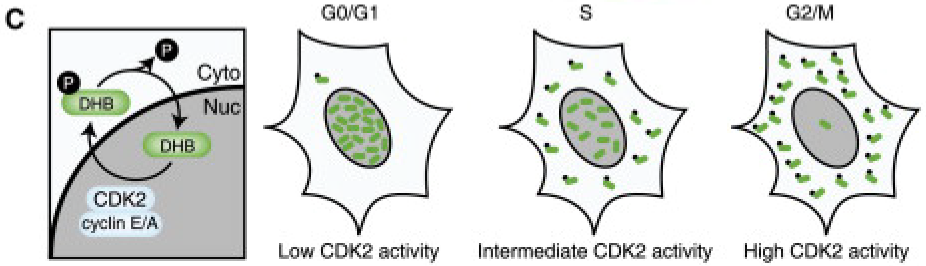
Schemes of biosensing

1. Presence or absence directly affects fluorescent molecule
2. Presence or absence causes a binding or conformational shift -> FRET change
3. Dimerization dependent biosensors
4. Location dependent sensors



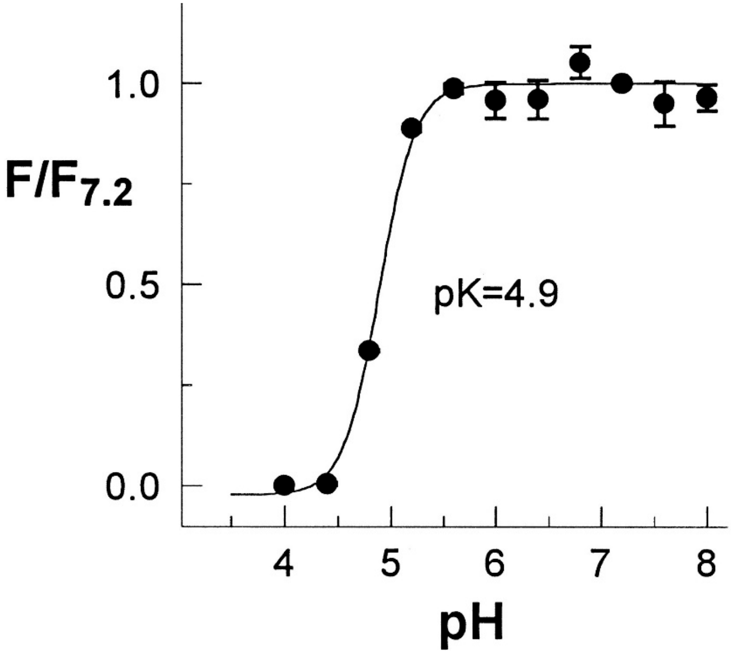




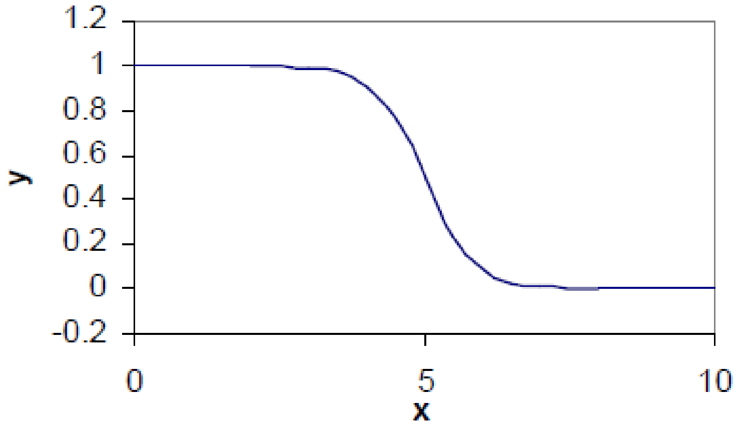


Basics of biosensors

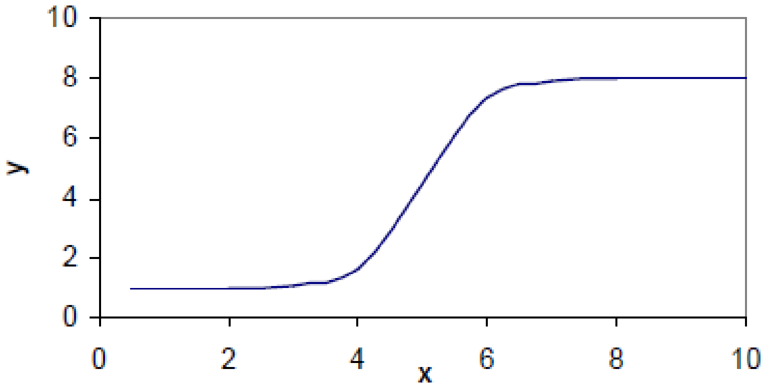
- Almost all sensors have hill function sensitivity
- Most sensitive range is at the K_d
- Any single molecule has some probability of being in each state



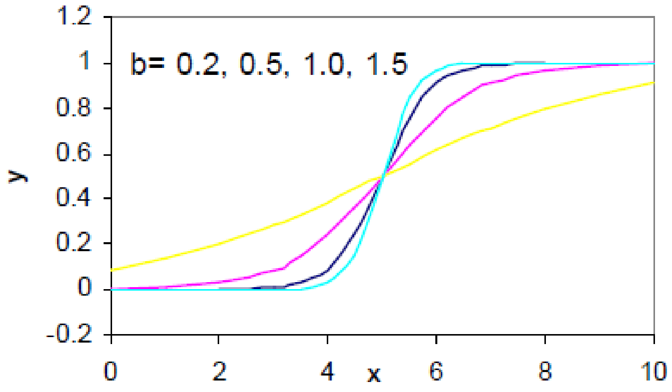
$$f = \frac{a}{1 + 10^{(x-b)}} + c$$



$$f = \frac{a}{1 + 10^{(b-x)}} + c$$



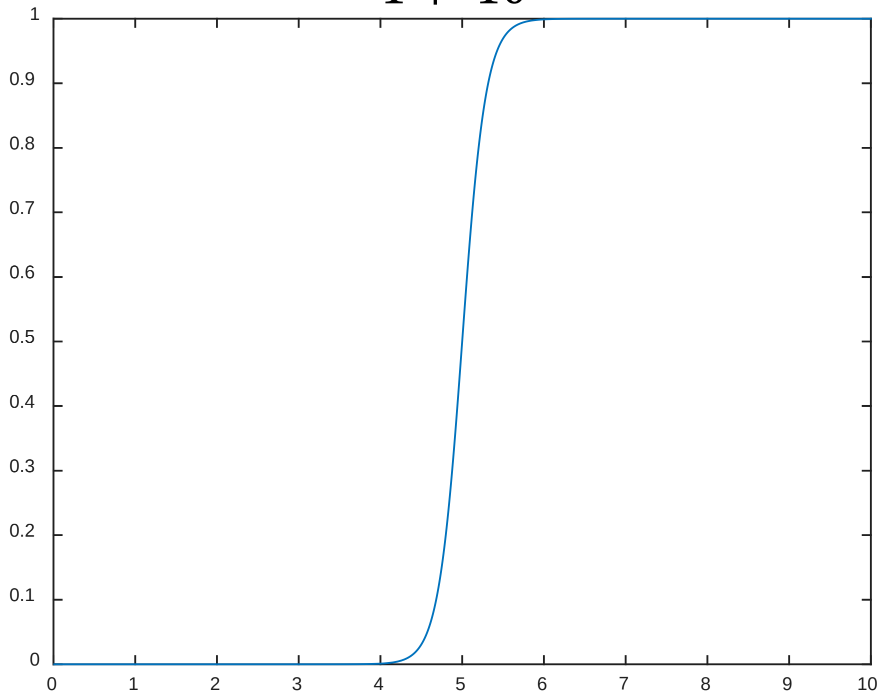
$$f = \frac{a}{1 + 10^{b(c-x)}}$$



Different regimes of sensing

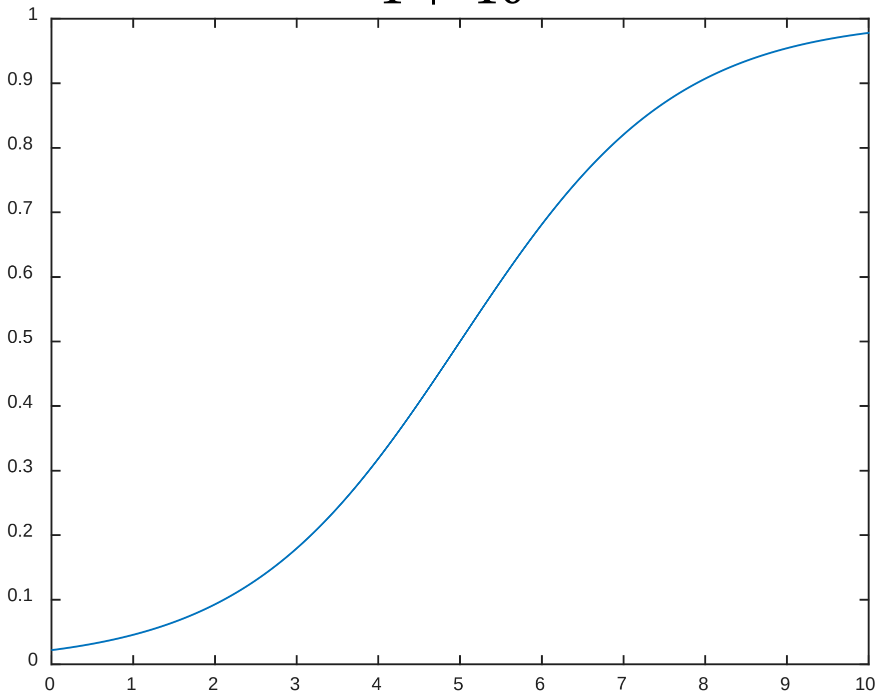
Binary change
Signal is on or off, but the signal to noise is VERY high

$$f(x) = \frac{1}{1 + 10^{3*(5-x)}}$$



Linear-ish change
Signal smoothly varies across entire range
Good for quantitating the actual quantity

$$f(x) = \frac{1}{1 + 10^{0.33*(5-x)}}$$



Basics of biosensors

- Dynamic range
- Response time
- Sensitivity

$$SNR = \frac{I_{obs} - I_{basal}}{I_{basal}}$$

Two ways to improve SNR:
Reduce I_{basal}
Increase I_{obs}

$$K_d = \frac{[A][B]}{[AB]}$$

$$v_f = v_r \text{ @ } equilibrium$$

$$k_f[A][B] = k_r[AB]$$

$$\frac{k_{on}}{k_{off}} = K_{eq} = \frac{1}{K_d}$$

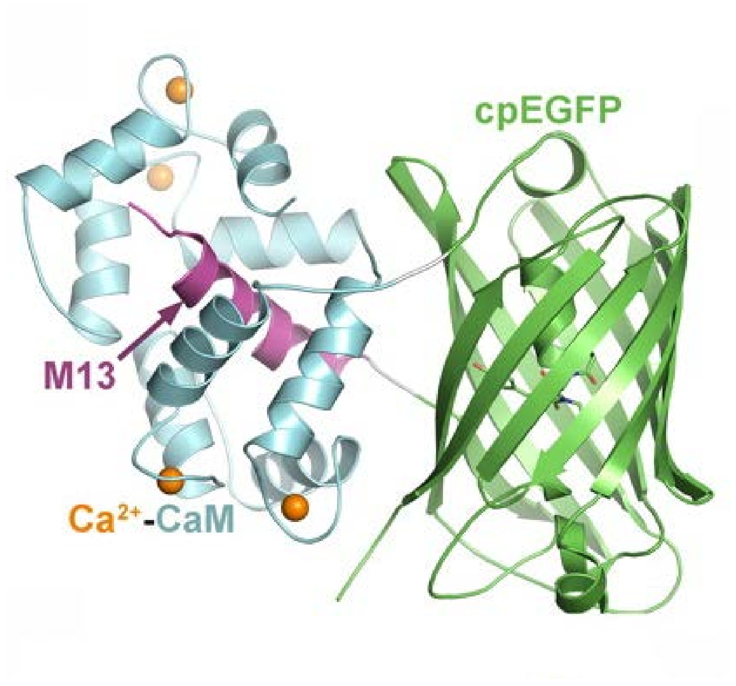
Complex	**KD (M)**	***koff*** **(s^{-1})**	***t1/2***
H_2	1×10^{-71}	1×10^{-63}	2×10^{55} yr
Avidin:biotin	10^{-15}	1×10^{-7}	80 days
lacrep:DNAoper(c)	1×10^{-13}	1×10^{-5}	0.8 days
Zif268:DNA(d)	10^{-11}	1×10^{-3}	700 s
GroEL:r-lactalbumin(e)	10^{-9}	0.1	7 s
LDH (pig): NADH(g)	7.1×10^{-7}(j)	7.1×10^{1}	10 ms
Creatine Kinase: ADP	8.2×10^{-4} (j)	$8.2\text{X}10^{4}$	10 ms
Acetylcholine:Esterase	1.2×10^{-3}	1.2×10^{5}	6 ms

Real life trade-offs

- GCaMP6 is a family of calcium sensors
- Physical limitation between sensor kinetics and sensitivity
- Fast sensors have higher binding coefficients

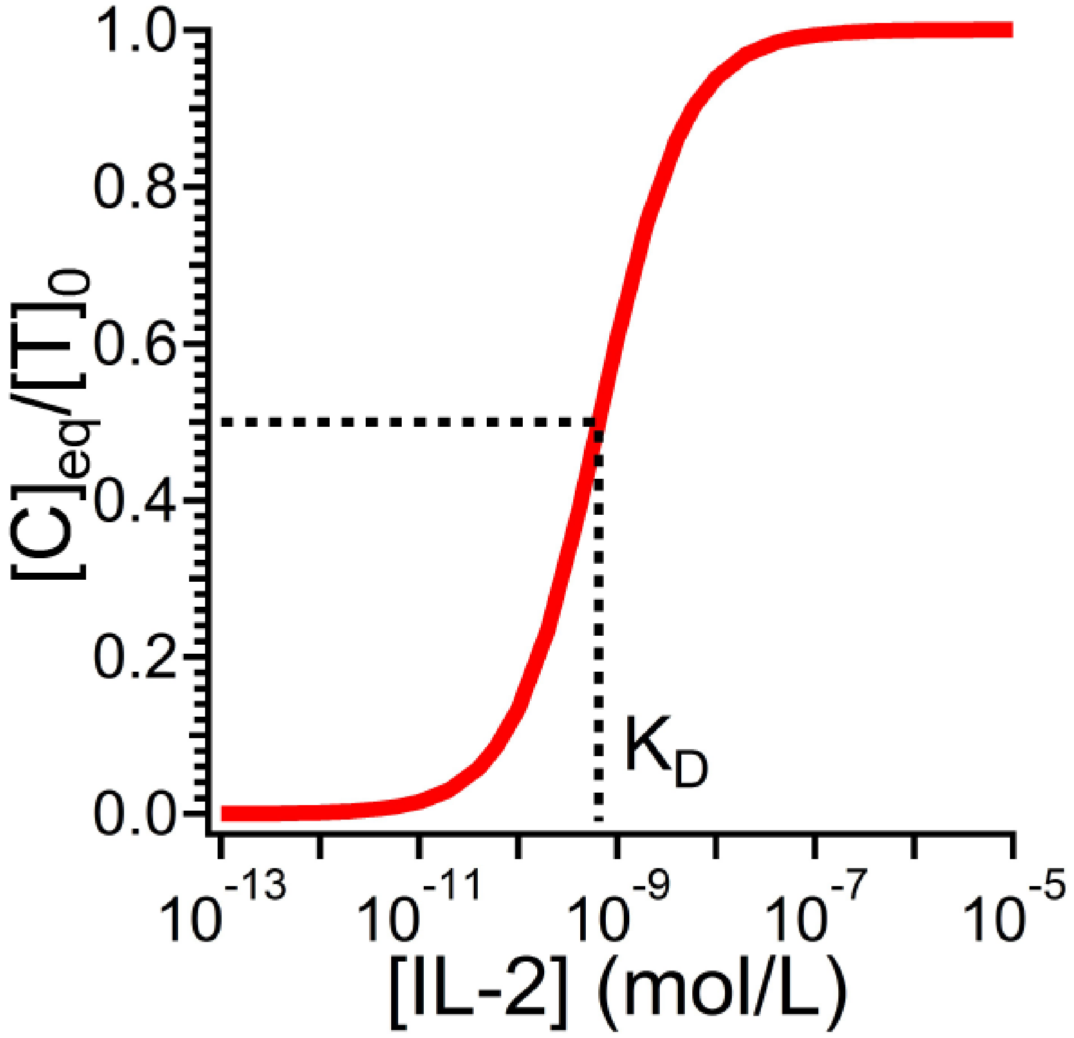
Sensor	K_d (nM)	k_{off} (s^{-1})
GCaMP3	345±17	2.57
GCaMP5G	447±10	2.52
GCaMP6s	144±4	1.12
GCaMP6m	167±3	2.06
GCaMP6f	375±14	3.93

Resting calcium concentrations:
Cytoplasm ~ 100 nM
Endoplasmic Reticulum ~ 200 μM
Mitochondria ~ 30 μM



Dynamic range, limit of detection, sensitivity

- LOD – lowest concentration at which a sensor can detect a clearly distinguishable signal (SNR > 1)
- Dynamic range - range of *reliable* detection concentrations
- Sensitivity – Smallest change in analyte concentration that can be *reliably* detected



Dangers of biosensors

- Does it over-compete for your analyte?
- Does it inhibit other cellular processes?
- Does it respond to other cellular components (pH!!, others)?
- Does it get sequestered by cellular machinery?
- Is the sensitivity range useful?

Calcium:
Free calcium – 80 nM
How many proteins do you need to express to buffer that [Ca]?

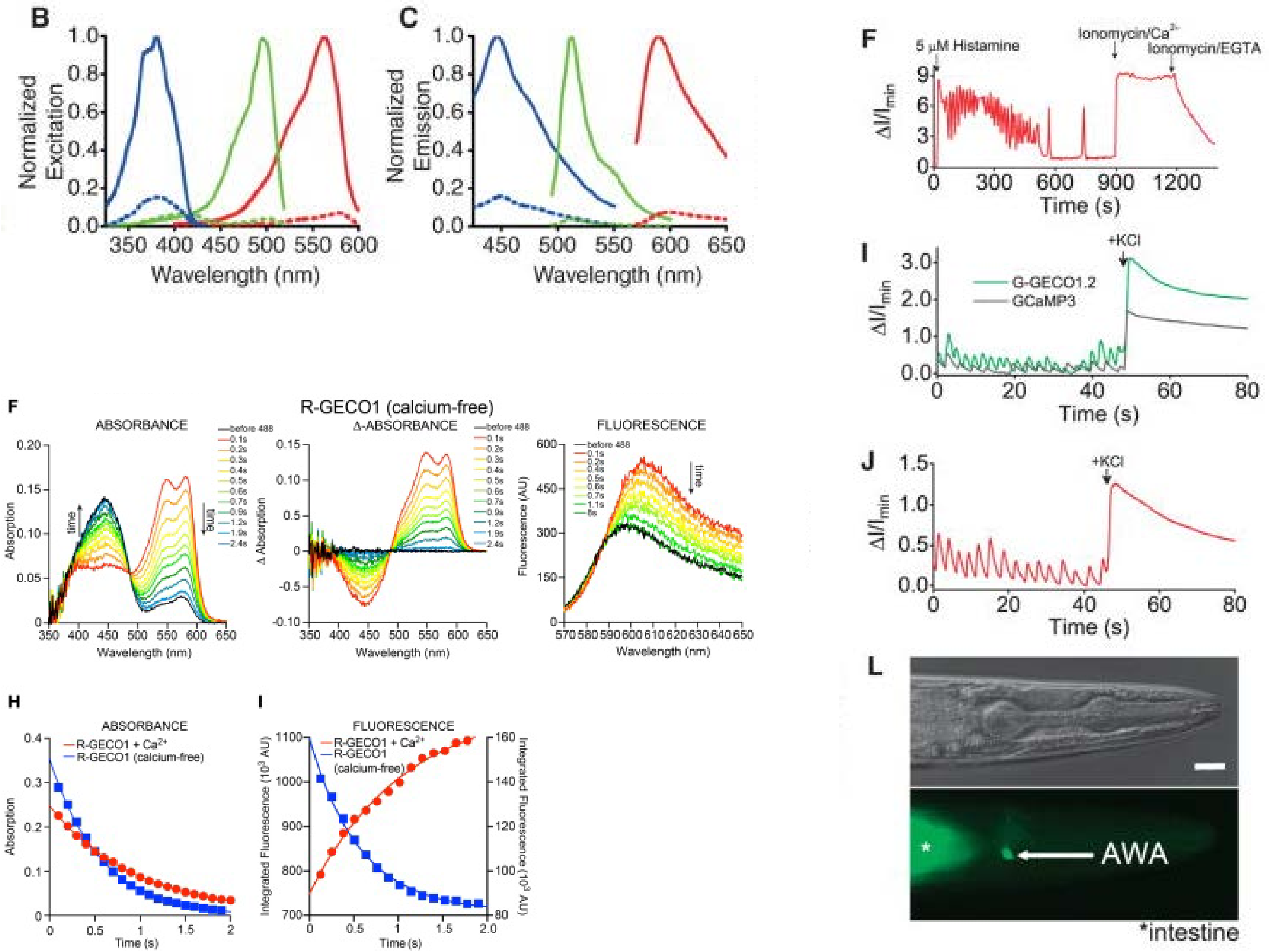
Protons:
pH 7.2
How many free protons in cells?

$$[H] = 10^{-7.2} = 63\ nM$$

Sensor	Dynamic range (F_{max}/F_{min})	K_d (nM)	Hill coefficient	k_{off} (s^{-1})	$pK_{a,\ apo}$	$pK_{a,\ sat}$
GCaMP3	13.5±0.7	345±17	2.54±0.04	2.57	8.44±0.01	7.13±0.07
GCaMP5G	45.4±0.9	447±10	2.46±0.04	2.52	8.61±0.15	6.58±0.02
GCaMP6s	63.2±3.1	144±4	2.90±0.17	1.12	9.77±0.70	6.20±0.02
GCaMP6m	38.1±1.8	167±3	2.96±0.04	2.06	8.68±0.09	6.90±0.04
GCaMP6f	51.8±2.8	375±14	2.27±0.10	3.93	8.77±0.16	6.34±0.01

Dangers of photophysics

- In 2011, there was a hunt for a good red calcium indicator
- Group A published in Science their sensor, RGECO
- One hope was to pair it with channelrhodopsin – blue light will stimulate cells, and read calcium transients with red



On to Matlab…