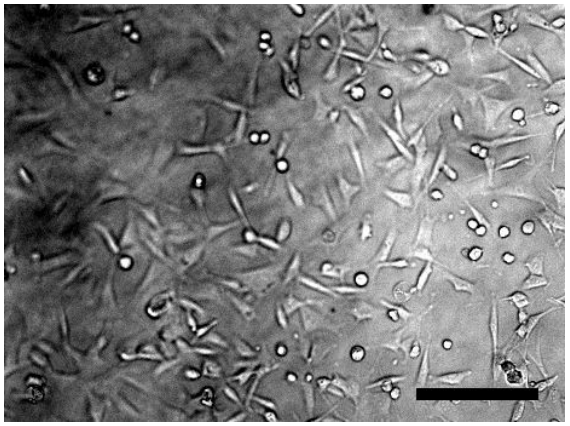
# Cell Viability; Standards in Scientific Communities I

Module 3, Lecture 3

20.109 Spring 2014

# Lecture 2 review

- What properties of hydrogels are advantageous for soft TE?



- What is meant by bioactivity and how can it be introduced?

- What are the two major matrix components of cartilage and how do they support tissue function?

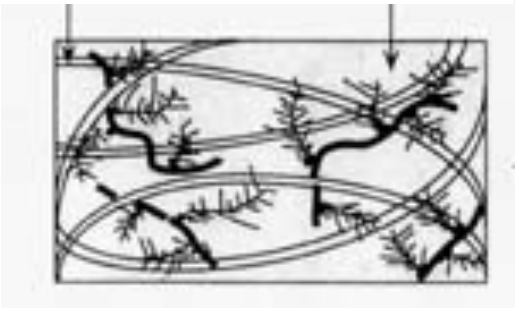


Image: VC Mow, A Ratcliffe, SLY Woo, eds *Biomechanics of Diarthrodial Joints* (Vol I). Springer-Verlage New York Inc., 1990.

# Module 3 learning goals

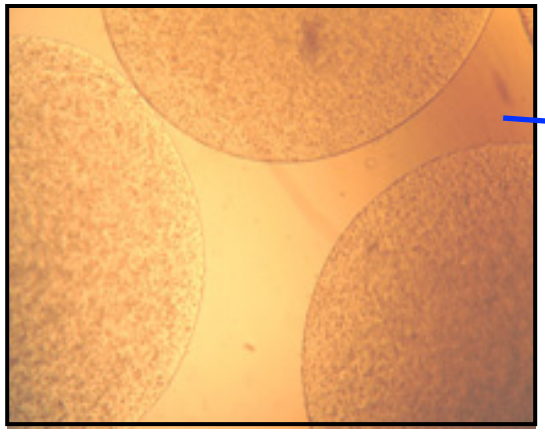
- Lab concepts/techniques
  - 3D mammalian cell culture and phenotypic assays
- Discussions in lecture
  - engage with meta-scientific issues, ethics, etc.
- Short informal report
  - accountability to 20.109 community
- Research idea presentation
  - investigate literature independently
  - exercise scientific creativity
  - design experiments to address a specific question

# Topics for Lecture 3

- Cell viability
  - measurement
  - contributing factors
- Standards in scientific communities
  - general engineering principles
  - standards in synthetic biology
  - standards in data sharing

# Module progress: week 1

- Day 1: culture design
  - What did you test?



- Day 2: culture initiation
  - Cells receiving fresh media every day
  - Half of volume exchanged, half kept

# Fluorescence microscope parts

- Light source
  - Epifluorescence: lamp (Hg, Xe)
  - Confocal: laser (Ar, HeNe)
  - 2-photon: pulsed laser
- Filter cube
  - Excitation
  - Dichroic mirror
  - Emission
  - Band-pass vs. long-pass
- Detection
  - CCD camera: photons → voltages → pixel intensities

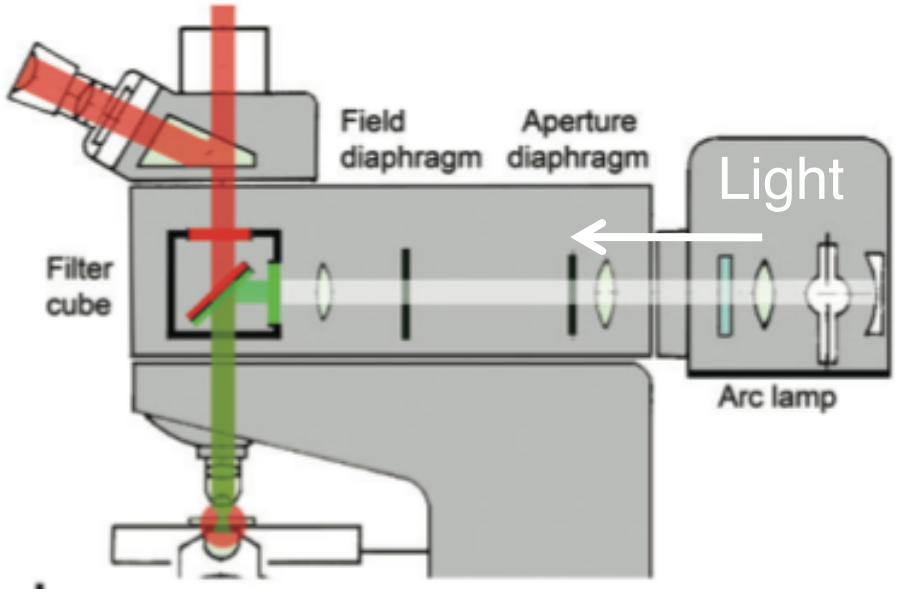
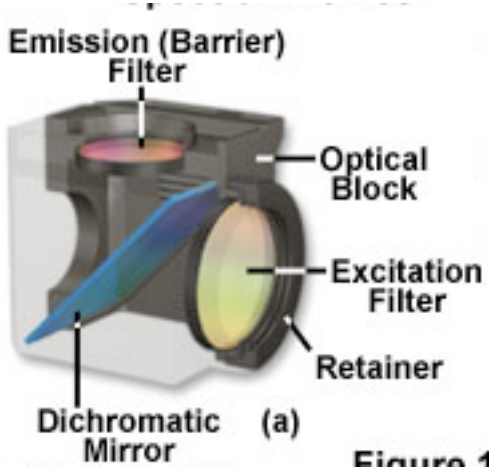
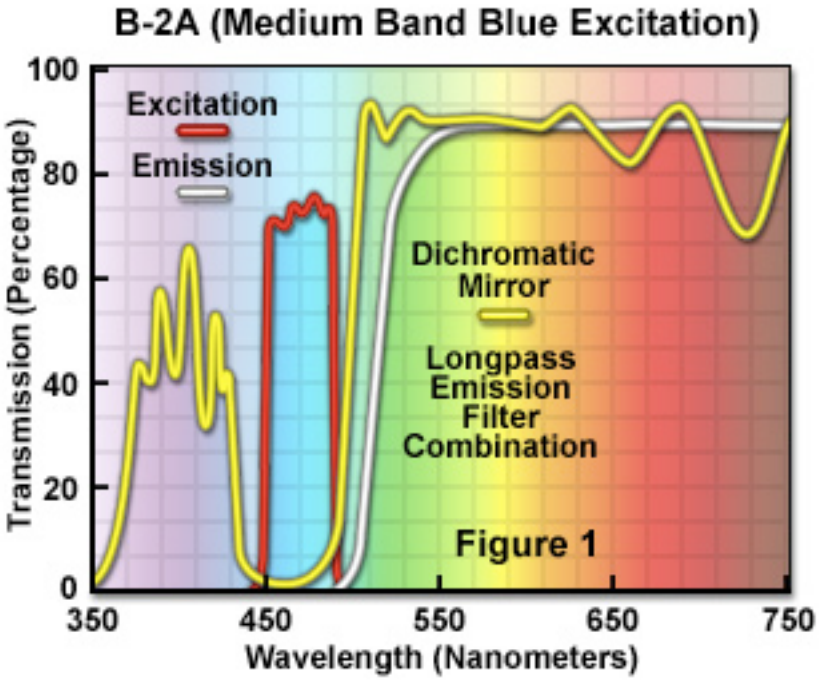



Image from: Lichtman & Conchello, *Nature Methods* 2:910 (2005)

# Specifications for M3D3 imaging

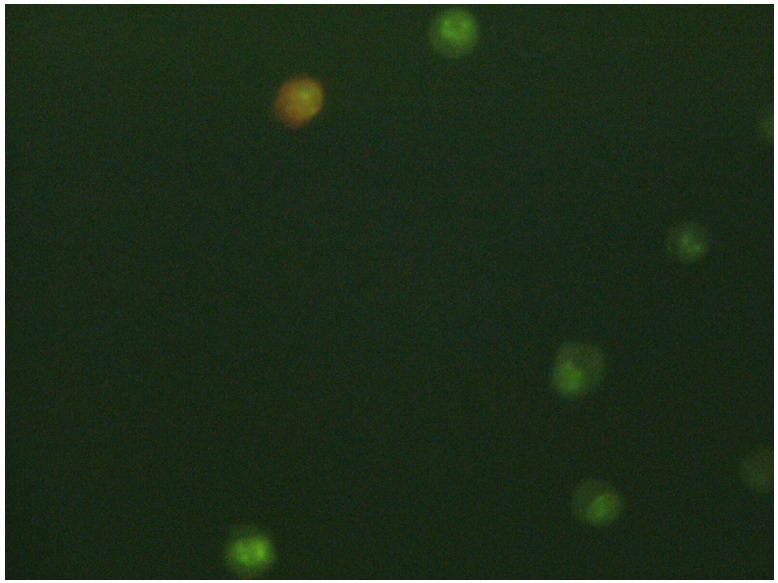
- Live/Dead Dyes
  - Green 490 ex, 520 em
  - Red 490 ex, 620 em
- Excitation 450-490 nm
- Dichroic 500 nm
- Emission $515^{+}$ nm

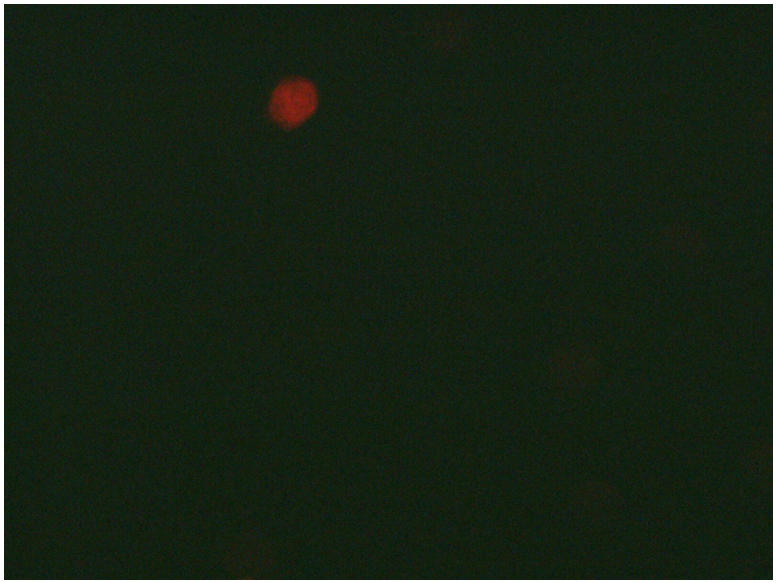



Images from: Nikon microscopy website: www.microscopyu.com

# M3D3 viability assay





Green stain: SYTO10 = viability
Red stain: ethidium = cytotoxicity

} Assay readout: fluorescence

Working principle? **Relative cell-permeability**

# Types of cell death

- Apoptosis
  - programmed cell death
  - role in development, immunity
  - cells condense, nuclei fragment
  - misregulation may cause disease
- Necrosis
  - response to trauma
  - cells burst and release contents
  - promotes inflammation
- Different morphology and biochemistry

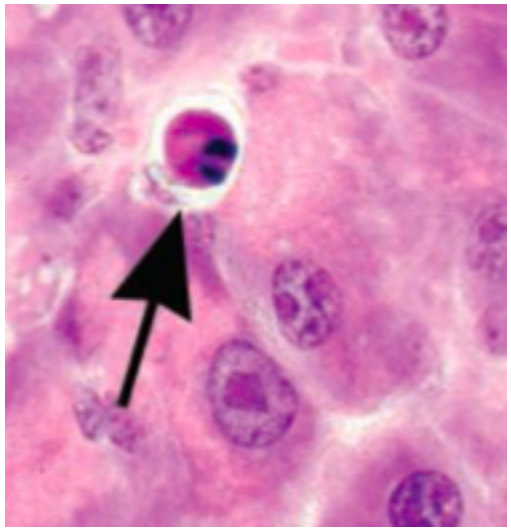
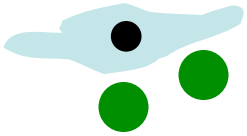
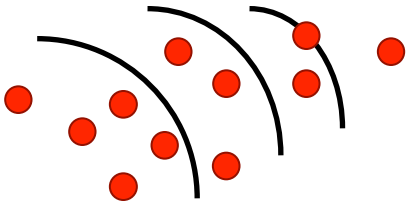


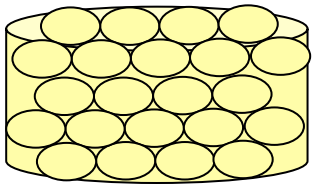
Image: S. Elmore *Toxicol Pathol* 35:495 (2007)

# Factors affecting cell viability

- Cell-related
  - density
  - contact
- Cytokine-related
  - proliferative
  - apoptotic
- Materials-related
  - bulk permeability
  - macro-porosity
  - toxicity

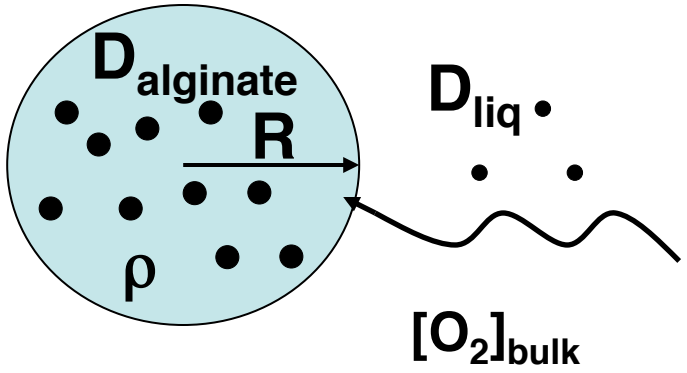


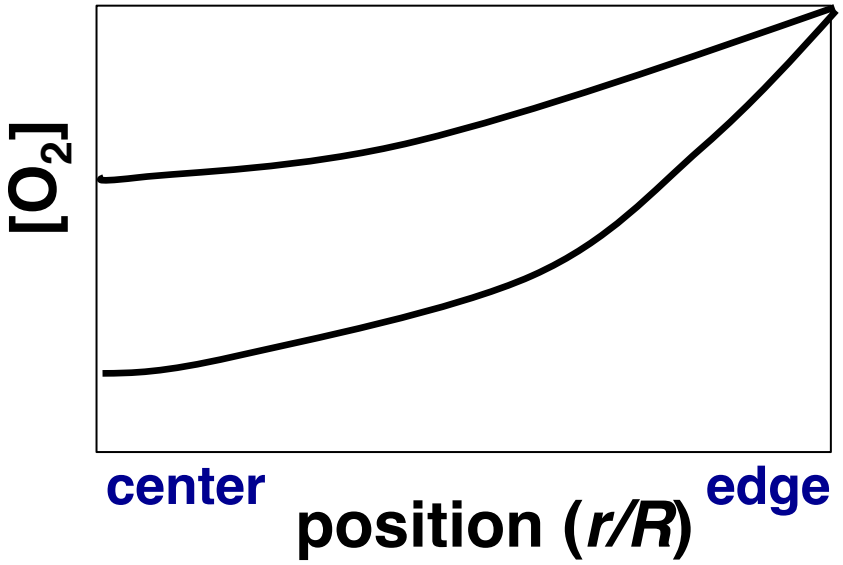




# Diffusion in 3D constructs

- Nutrients and $O_2$
- Affected by
  - construct size R
  - cell density $\rho$
  - diffusivity D
  - conc. in medium $[O_2]_{bulk}$
- Concentration profile
  - can be solved Diff-Eq
  - [O2] ↓ toward center
  - steepness = f(D, $\rho$, ...)

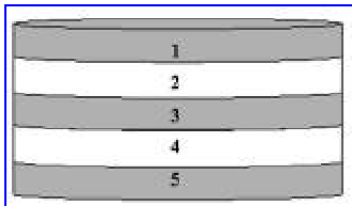


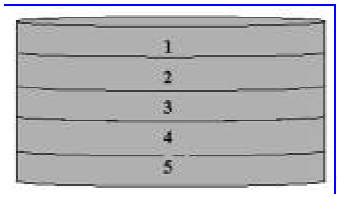
# Modeling cell viability in TE constructs

- Porous PLGA scaffolds
- Seeded cells as in (A) or (B)
- Observed after 10 days
- Model includes
  - Diffusion
  - $O_2$ use
  - Cell growth
- Model assumes
  - $[O_2]_{bulk}$ is constant
  - Quasi-steady state

**A Cells in odd layers**



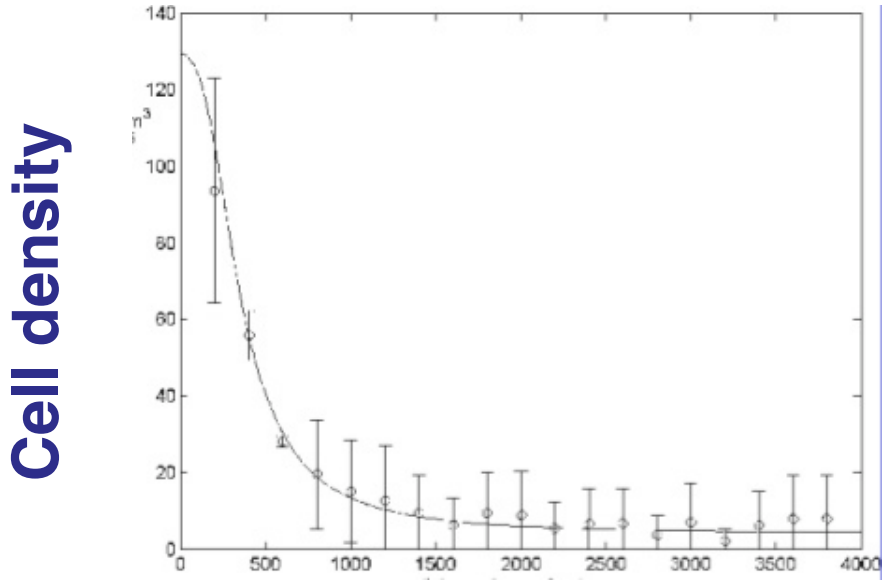

**B Cells in all layers**

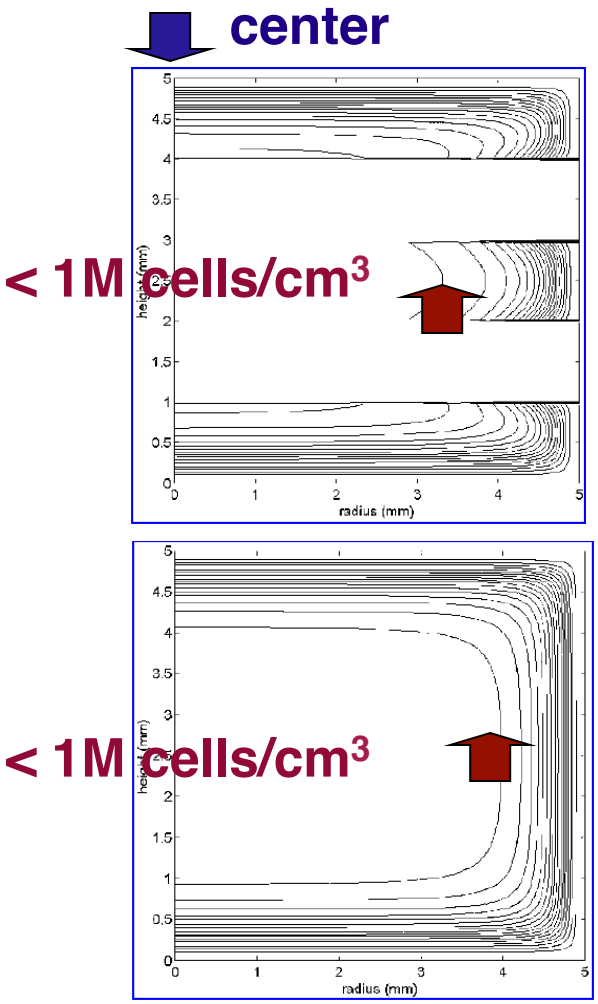



J Dunn, et al. *Tissue Eng* **12**:705 (2006)

# Viability model and experiment

- A more uniform than B
- Cell growth matches $O_2$ tension
- Claim of predictive capability



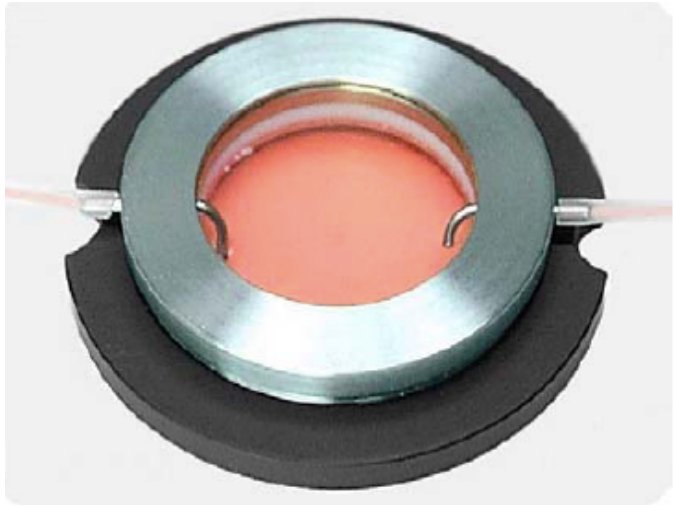

Dunn, et al.

# Significance of diffusion in TE

- Characteristic limit ~100 μm
- Diffusion and viability profiles correlated
- How can we make thick tissues?

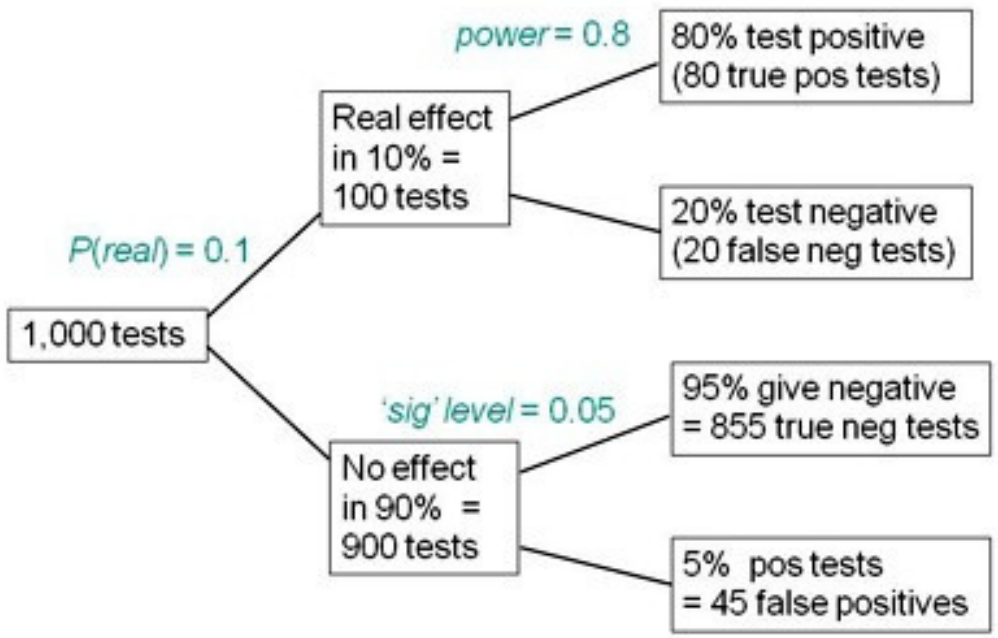
  *in vitro*: dynamic/perfusion culture

  *in vivo*: promote rapid angiogenesis



perfusion system
zeiss.com.sg

# Interlude: limitations of the p-value



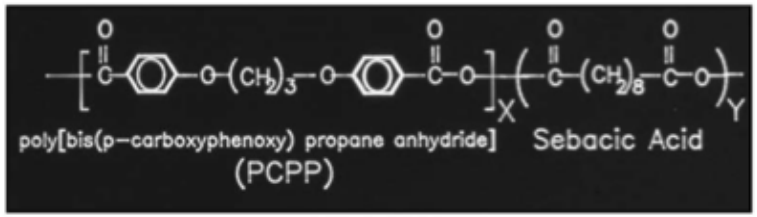

Total number of positive tests = 80 + 45

False discovery rate (proportion of false positives) $\frac{45}{45+80} = 36$ percent (NOT 5%)

**David Colquhoun** via mikethemadbiologist.com2014/04/10/p-values-and-power-of-test-why-so-many-results-cant-be-replicated/

# Thinking critically about module goals

- Local: compare 2 culture conditions → cell phenotype?
- Global: toward cartilage tissue engineering
- All well and good, but…
- Can we move beyond empiricism – tissue *engineering*
- Broadly useful biomaterials example
  - goal: wide degradation range
  - result: times from weeks to years
  - process: models and experience




"a lot of chemical calculations later, we estimated that the anhydride bond would be the right one"

Image and quote: Robert Langer, *MRS Bulletin* **31** (2006).

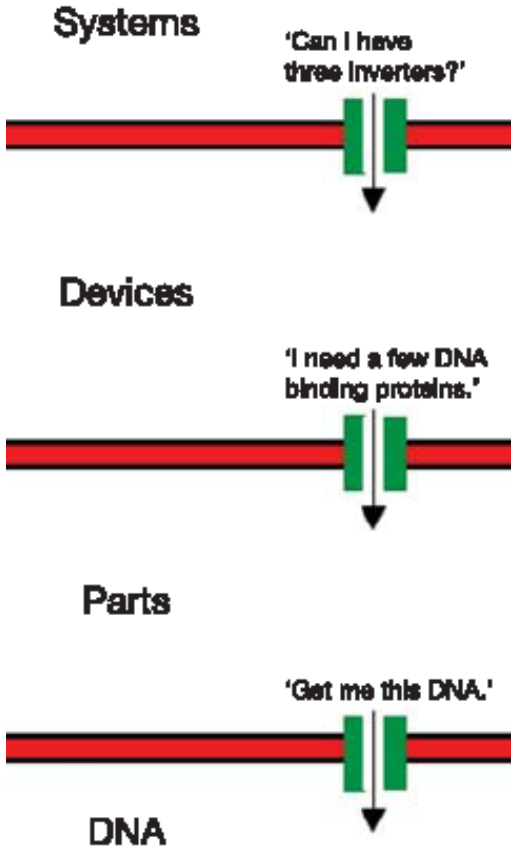
# Biology: too complex to engineer?

- Systematic vs. *ad hoc* approach
- D. Endy, *Nature* **438**:449 (2005)
- Need for "foundational technologies"
- Decoupling
  - e.g., architecture vs. construction
- Abstraction
  - e.g., software function libraries
- Standardization
  - screw threads, train tracks, internet protocols
- What can and/or should we make standard to engineer biology?


Public domain image
(Wikimedia Commons)

# Apply principles to synthetic biology

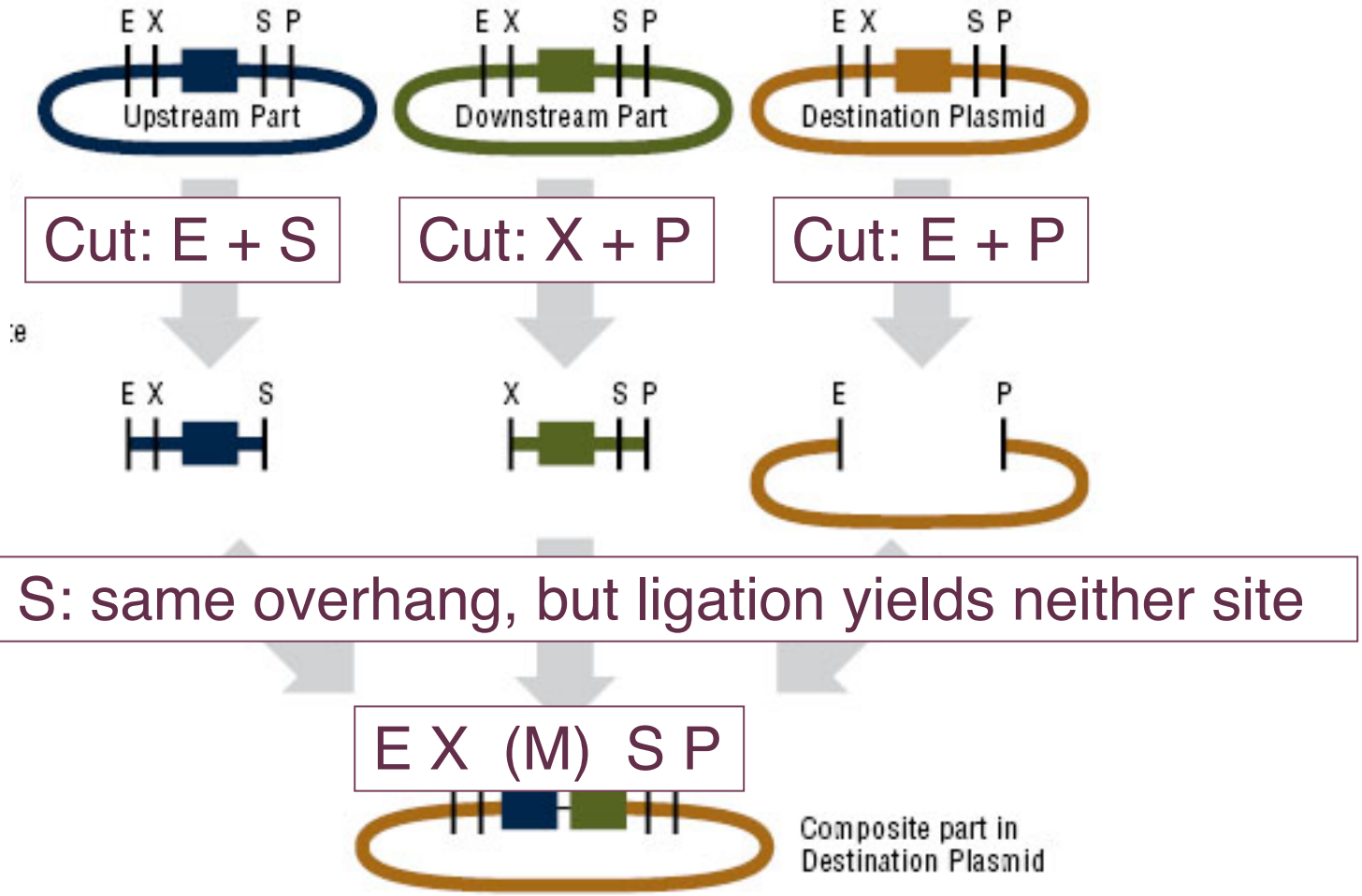
- Synthetic biology, in brief: "programming" cells/DNA to perform desired tasks
  - artemisinin synthesis
  - genetic circuit
- Decoupling
  - DNA design vs. fabrication (rapid, large-scale)
- Abstraction
  - DNA → parts → devices → systems
  - materials processing to avoid unruly structures
- Standardization
  - standard junctions to combine parts
  - functional (e.g., RBS strength)
  - system conditions
  - assays




D. Endy, *Nature* **438**:449 (2005).

Not fair game for quiz

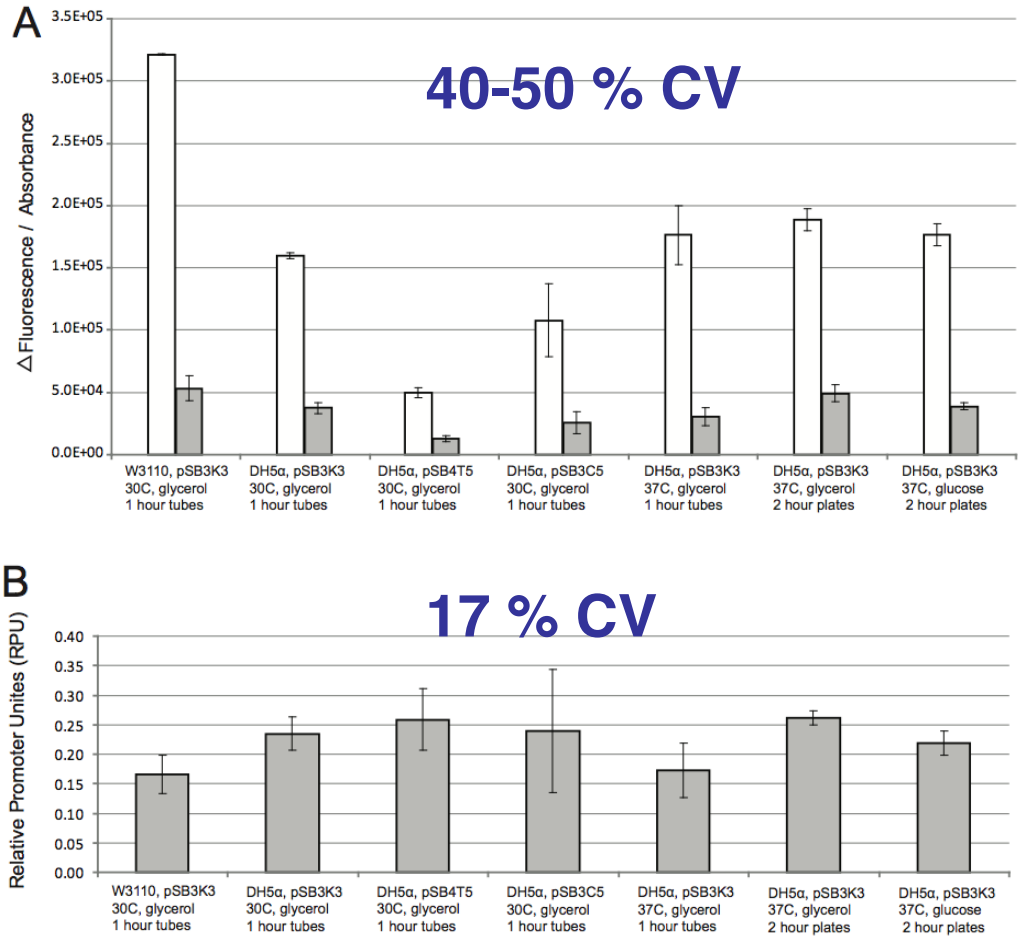
# Assembly standard for plasmids




Development: T.F. Knight, R.P. Shetty, D. Endy; Image: neb.com

Not fair game for quiz

# Functional standard for promoters




**Absolute promoter strength**

Variation due to cell strain, equipment, media, lab, etc.

(white & grey = 2 promoters)

**Relative promoter strength**

Variation reduced 2-fold.

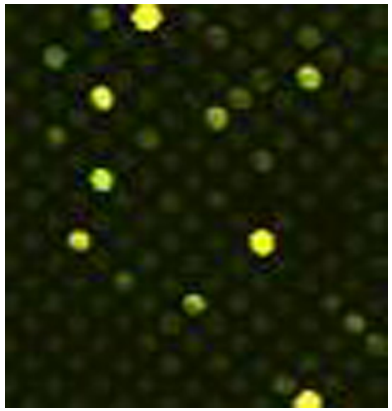
(same 5' UTR)

J.R Kelly et al., *J Biol Eng* **3**:4 (2009)

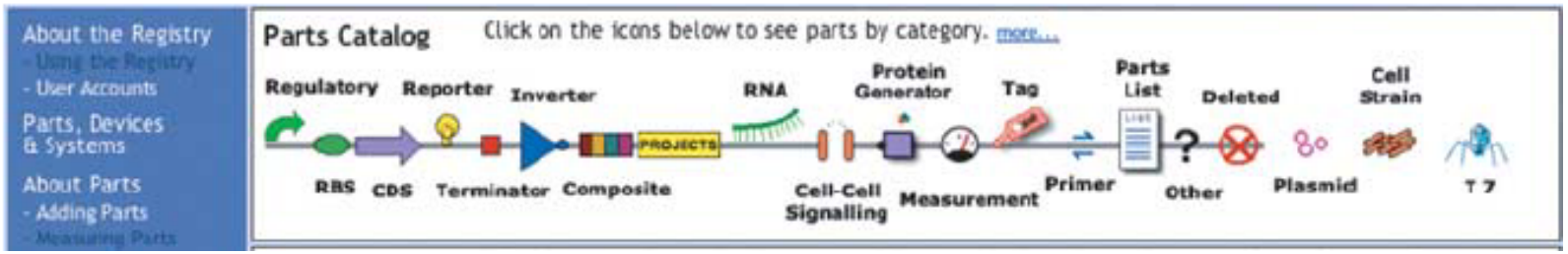
# Lecture 3: conclusions

- Cell viability in TE constructs is affected by cell, material, and soluble factors.
- Standardizing data sharing and collection is of interest in several BE disciplines.

Microarray data



From D. Endy, *Nature* **438**:449 (standardized biological "parts")




Next time: TE-specific lecture and *discussion* of standards.