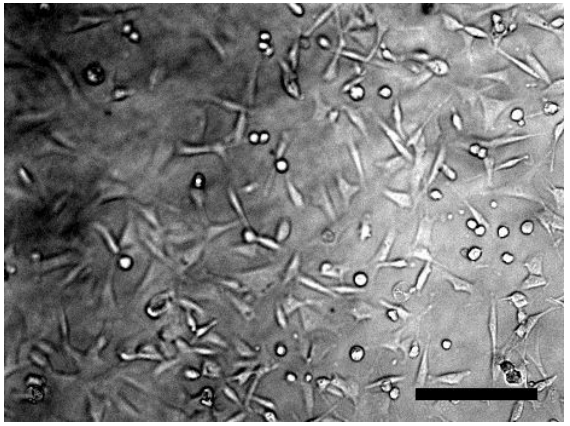
Basic Statistics; Standards in Scientific Communities I

Module 3, Lecture 3

20.109 Spring 2012

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?



Principles of osmotic pressure

- Water must have equal chemical potential in both compartments: $\mu_{H2O,1} = \mu_{H2O,2}$
- If water is constrained from entering compartment 1 to equalize solute concentrations, an excess pressure in compartment 1 makes for equal μ
- Charges must also be balanced (Donnan equilibrium)

Simplified cartilage model

Membrane: solutes cross slower (or not at all) than H_2O →

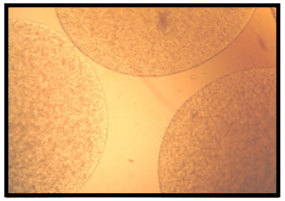
H_2O + high $[Na^+]/PG^-$	**1**
H_2O + low $[Na^+]$	**2**

Topics for Lecture 3

- Module 3 so far, and Day 3 plan
- Introduction to statistics
  - confidence intervals
  - t-test
- Standards in scientific communities
  - general engineering principles
  - standards in synthetic biology
  - standards in data sharing

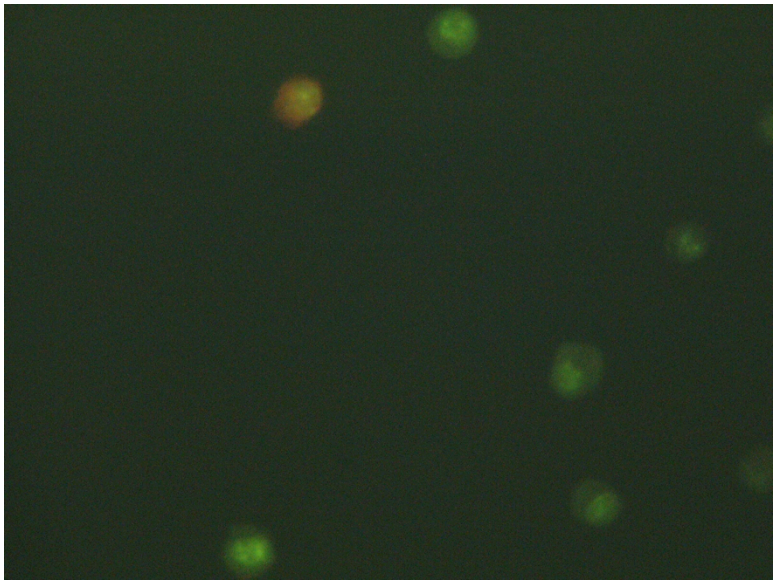
Module progress: week 1

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



- Day 2: culture initiation
  - Cells receiving fresh media every 2 days

Module day 3: test cell viability



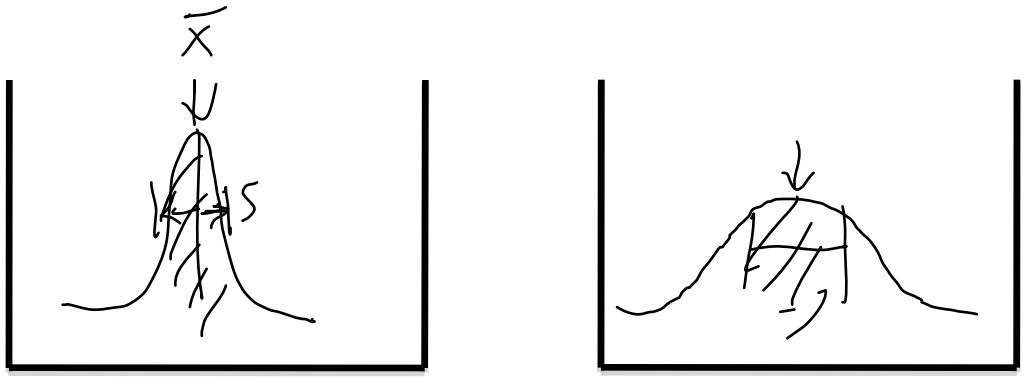
Green stain: SYTO10 = viability
Red stain: ethidium = cytotoxicity

} Assay readout: fluorescence

Working principle? **Relative cell-permeability**

Statistics review: basics

- Essential concepts: standard deviation (*s*), mean ($\bar{x}$), sample size *n,* degrees of freedom *DOF*
- Normal (Gaussian) distribution



1 *s* includes

68 %

of the data

x-axis: measured values (intensity)

y-axis: # of samples w/ that value

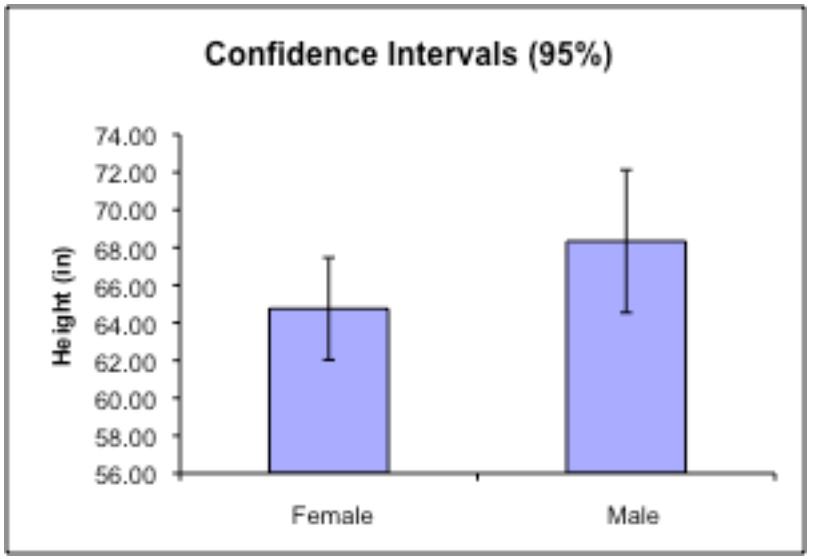
Confidence intervals (CI): principle

- $\overline{x}$ = 60 (sample/measured mean)
- 95% CI calculated to be ± 3 from real data
- Thus: 95% of the time our population (true) mean μ lies in the range, here 60 ± 3
  - subtly different from 95% likely that the range 60 ± 3 contains the population (true) mean μ, which we can't say
- 90% CI: $\mu = \overline{x} \pm a$ where $\boldsymbol{a < 3}$ $a > 3$ $a = 3$?

  trade-off between precision and confidence
- Consider betting example
- What about n? **as n increases, more precise**

Calculating confidence intervals (CI)

$$\mu = \bar{x} \pm \frac{t\,s}{\sqrt{n}}$$



- *t* is tabulated by DOF vs CI%
  - DOF = *n* - 1 (why? $\sum(\text{errors} = x_n - \bar{x}) = 0$ → constraint)
- In Excel, us *TINV* function
  - input *p*-value = (100-CI)/100

CI = 95%, $p = 0.05$

Introduction to t-test

- Every statistical test
  - has assumptions
  - asks a specific question
  - requires human interpretation
- Some t-test assumptions
  - normal distribution (cf. Mann-Whitney test)
  - equal variances (type 2 in Excel; type 3 unequal)
- Posing a question *are average male and female heights different at a confidence level of 95%*

Calculating t-test significance

$$t_{calc} = \frac{\bar{x}_1 - \bar{x}_2}{S_{pooled}} \sqrt{\frac{n_1 n_2}{n_1 + n_2}}$$

DOF = $n_1 + n_2 - 2$

t_{table} listed by DOF vs. C.L.

- If $t_{calc} > t_{table}$ difference *is* significant at that C.L.
- In Excel, us *TTEST* function
- Excel returns *p*-value → confidence level (CL)
  $p = 0.01$ C.L. 99%
- 1-tailed vs. 2-tailed test
  1- one-sided hypothesis in advance
  2- no a priori hypothesis

Assignment for report

- Get live cell count and/or live cell percent values for both culture conditions
- Calculate 95% CI for both means
- Plot means on bar graph with CI error bars
- Apply t-test to the means
  - For multiple comparisons, ANOVA is better
  - Comparing many means requires correction
  - Remember, $p = 0.05$ means 1 in 20 false positives!

Interlude: perceptions of scientific progress

Read the highlighted excerpts from Chapter 7 of The Immortal Life of Henrietta Lacks

What scientific advances today bear a resemblance – in the hopes and/or fears they provoke – to tissue culture in the early 1900's? Does the TC historical perspective change your own thoughts or feelings about the promises and/or perils of current advances in science and technology? What role do scientists play in contributing to or correcting hype?

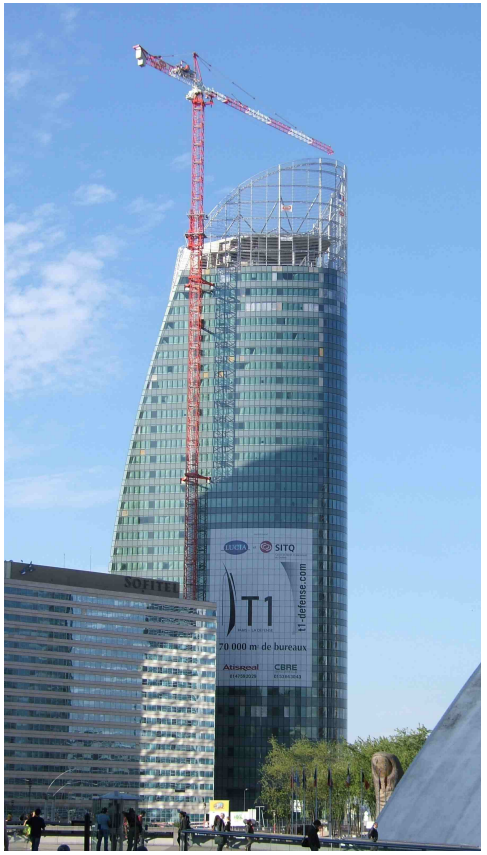
What moral responsibility do scientists have when they are speaking outside their domain but may be seen as experts?

Thinking critically about module goals

- Purpose of experiment
  - Local: compare 2 culture conditions → effect on cell phenotype
  - Global: toward cartilage tissue engineering
- All well and good, but…
- Can we move beyond empiricism – tissue *engineering*
- E.g., broadly useful biomaterials
  - goal: control degradability over wide range
  - "a lot of chemical calculations later, we estimated that the anhydride bond would be the right one"
  - achieved degradation times of weeks to years
  - Robert Langer, *MRS Bulletin* **31**(2006).

Engineering principles, after D. Endy

- D. Endy, *Nature* **438**:449 (2005)
- Is biology too complex to engineer, or does it simply require key “foundational technologies”?
- Systematic vs. *ad hoc* approach
- Abstraction
  - e.g., software function libraries
- Decoupling
  - e.g., architecture vs. construction
- Standardization
  - screw threads, train tracks, internet protocols
- What should and/or can we standardize to engineer biology?



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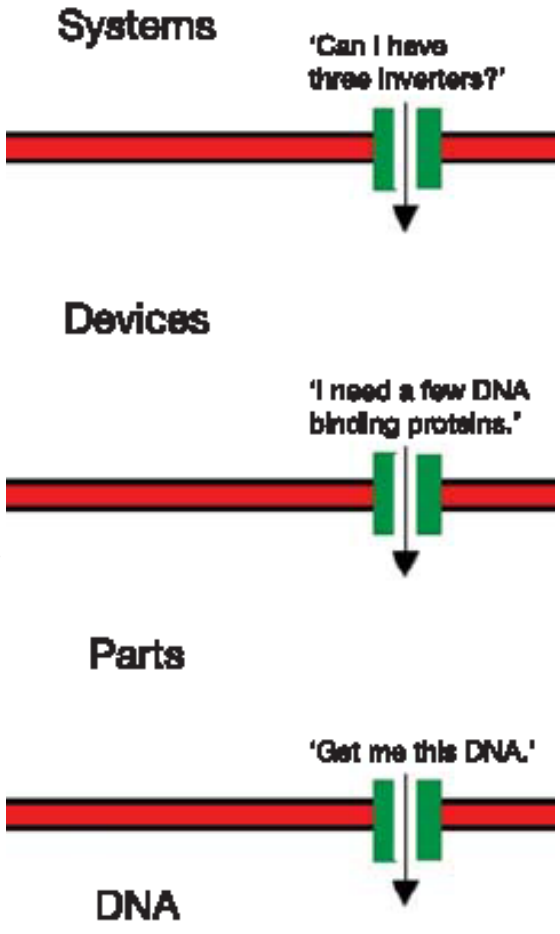
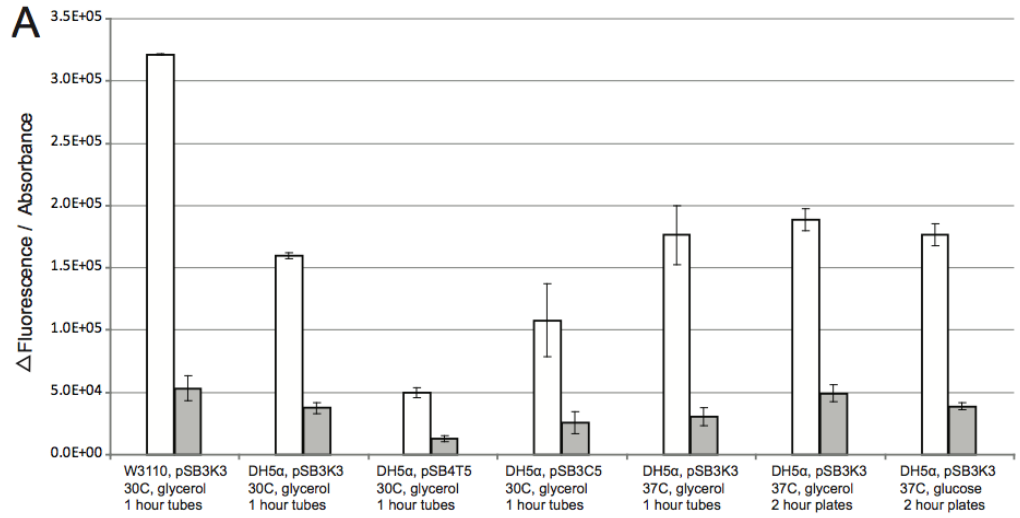
Application to synthetic biology

- D. Endy, *Nature* **438**:449 (2005)
- Synthetic biology, in brief: "programming" cells/DNA to perform desired tasks
  - artimisinin synthesis in bacteria
  - genetic circuits
- Abstraction
  - DNA → parts → devices → systems
  - materials processing to avoid unruly structures
- Decoupling
  - DNA design vs. fabrication (rapid, large-scale)
- Standardization
  - functional (e.g., RBS strength)
  - assays
  - system conditions
  - standard junctions to combine parts



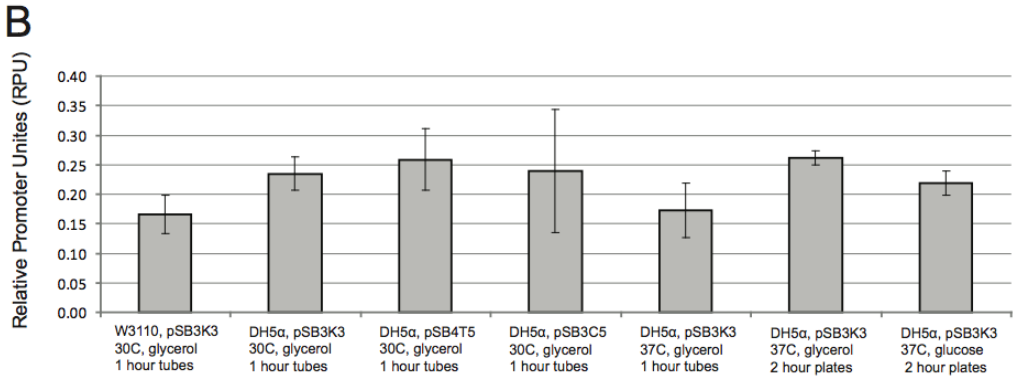
From D. Endy, *Nature* **438**:449

Advances in standardized parts



Absolute promoter strength

Measurement varies widely (cell line, equipment, etc.)



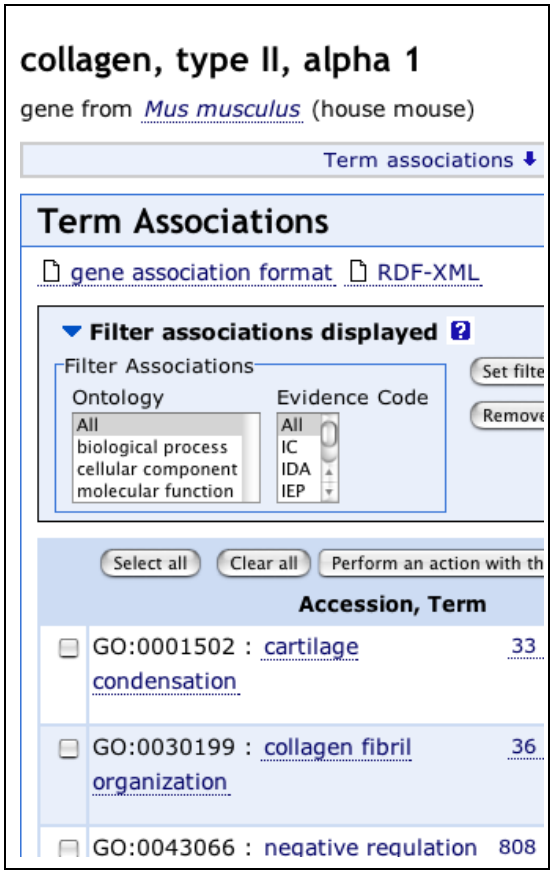
Relative promoter strength

Measurement less varied

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

Data standards: what and why?

- Brooksbank & Quackenbush, *OMICS*, **10**:94 (2006)
- High-throughput methods are data-rich
- Standards for **collection** and/or **sharing**
- Reasons
  - shared language (human and computer)
  - compare experiments across labs
  - integration of information across levels
  - avoid reinventing the wheel (save t, $)
- Examples
  - MIAME for microarrays
  - Gene Ontology (protein functions)
- Who drives standards?
  - scientists, funding agencies, journals, industry



www.geneontology.org

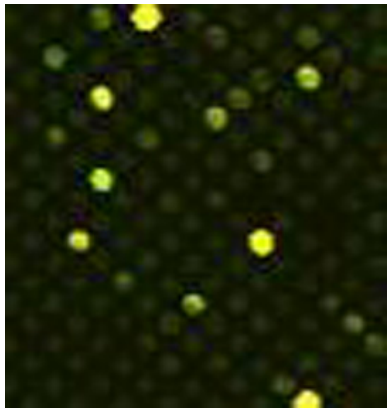
Standards: possible directions, considerations

- Role of biocuration [D. Howe et al., *Nature*, **4**:47 (2008)]
  - growth in data not yet causing equivalent growth in knowledge
  - need human element: catch errors, parse literature, develop systematic and useful shared vocabularies
  - curators must possess unique skills, but lack career incentives
  - creative uses of the public possible (cf Galaxy Zoo)
- Semantic Web concept [A. Ruttenberg et al., *BMC Bioinformatics*, **8(Suppl 3)**:S2 (2007)]
  - systematically define relationships with universally recognized tags
  - example: gene hasgeneproduct mRNA (3-part statements)
  - potential for automatic error recognition
  - potential for hiding complexity (data formats)

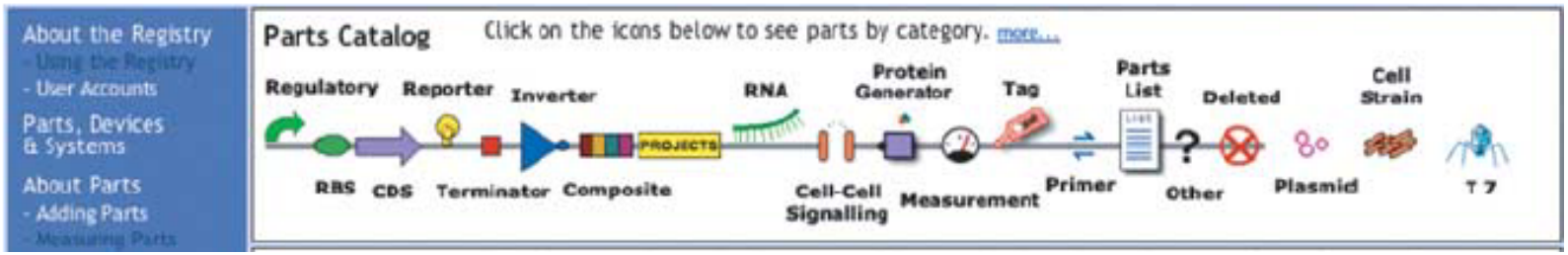
Lecture 3: conclusions

- Confidence intervals and t-tests are two useful statistical concepts.
- 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: *discussion* of standards in TE; more about cell viability and microscopy