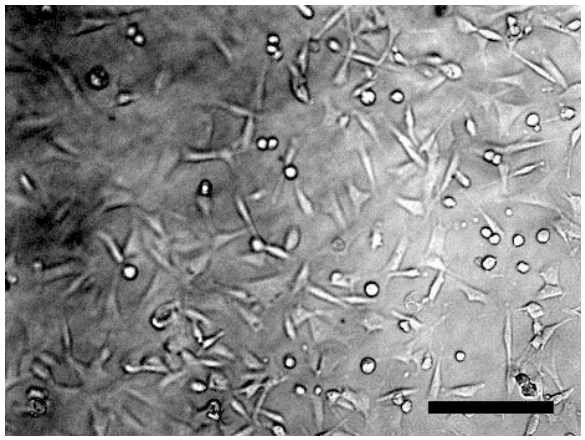
Basic Statistics; Standards in Scientific Communities I

Module 3, Lecture 3

20.109 Spring 2010

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?

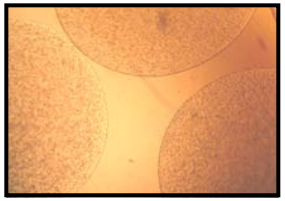


Topics for Lecture 3

- 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?



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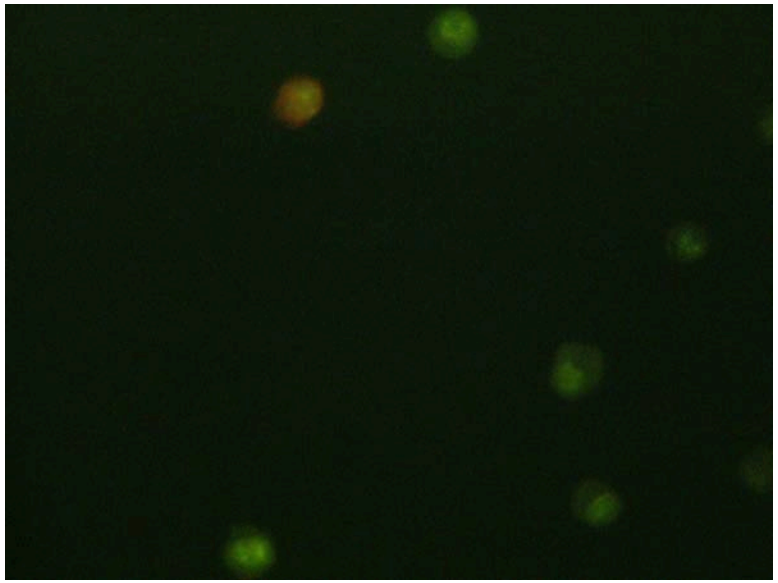
- pressure (compression)
- high + low pH conditions
- amount of x-linking [$CaCl_2$]

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

Module day 3: test cell viability



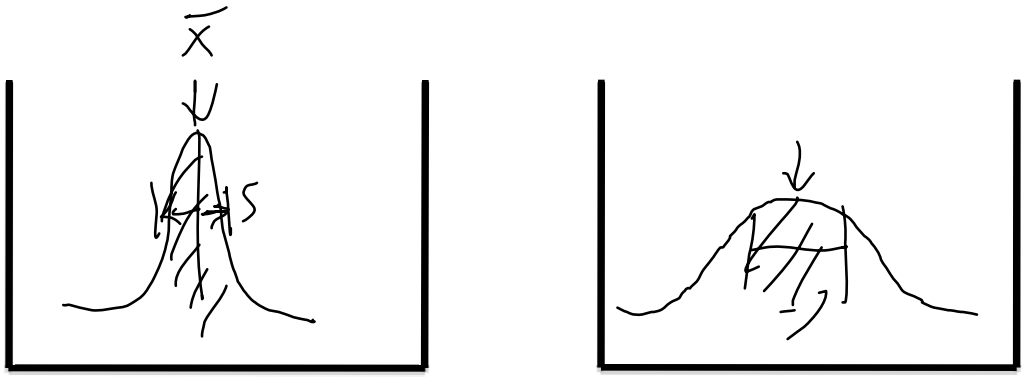
Green stain: SYTO10 = viability
Red stain: ethidium = cytotoxicity

} Assay readout: fluorescence

Working principle? **Relative cell-permeability**

Statistics review: basics

- Essential concepts: standard deviation (*s*), mean ($\overline{x}$), 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

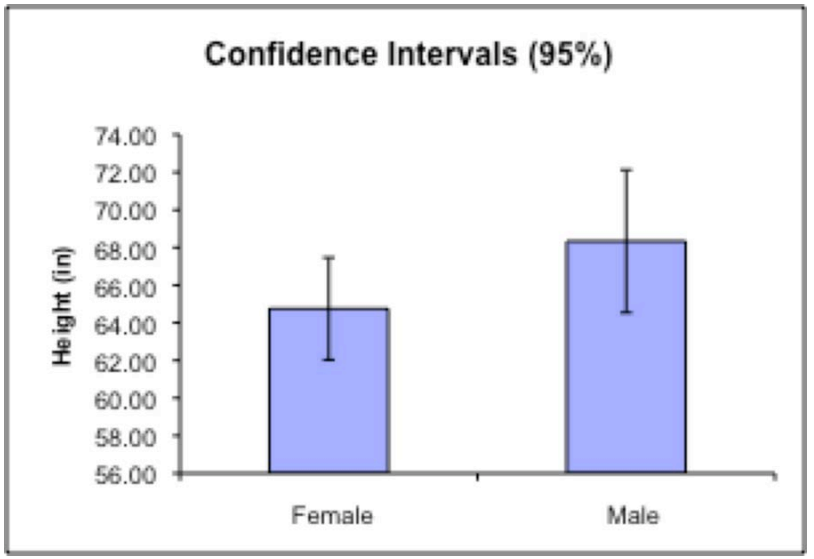
Confidence intervals (CI): principle

- $\bar{x}$ = 60 (sample/measured mean)
- 95% CI calculated to be ± 3
- Thus: 95% likely that the range 60 ± 3 contains the population (true) mean μ
  - exact definition is subtle
- 90% CI: $\mu = \bar{x} \pm a$ where $a < 3$ $a > 3$ $a = 3$?

  trade-off precision + confidence
- Consider betting example
- What about n? as n↑, 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
- In Excel, us *TINV* function
  - input *p*-value = (100-CI)/100

why? $\sum(\text{errors} = x_n - \bar{x}) = 0$ → constraint

C.I. = 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)
- Question are 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)
- 1-tailed vs. 2-tailed test

$p = 0.01$, C.L. 99%

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: intersection of science and commerce

1. HeLa cells

   http://www.colbertnation.com/the-colbert-report-videos/267542/march-16-2010/rebecca-skloot (~00:30-3:00)

2. Patenting genes
   "Judge invalidates human gene patent"
   NY Times March 2010
   "Metastasizing patent claims on BRCA1"
   Genomics May 2010

Thinking critically about module goals

- Purpose of experiment
  - Local compare 2 culture conditions ⇒ effect on cell phenotype
  - Global cartilage regeneration
- 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"
  - 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
  - software function libraries
  - copy-editor vs. editor
- Decoupling
  - architecture vs. construction
  - design vs. fabrication
- Standardization
  - screw threads, train tracks, internet protocols
  - what would we standardize to engineer biology?

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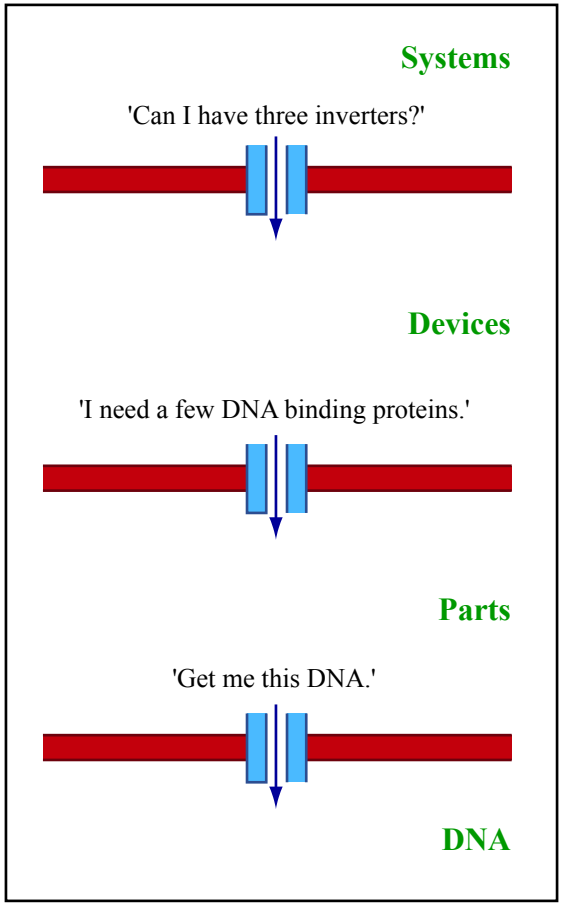
Application to synthetic biology

- 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
  - Registry of Standard Biological Parts
  - standard junctions, off-the-shelf RBS, etc.



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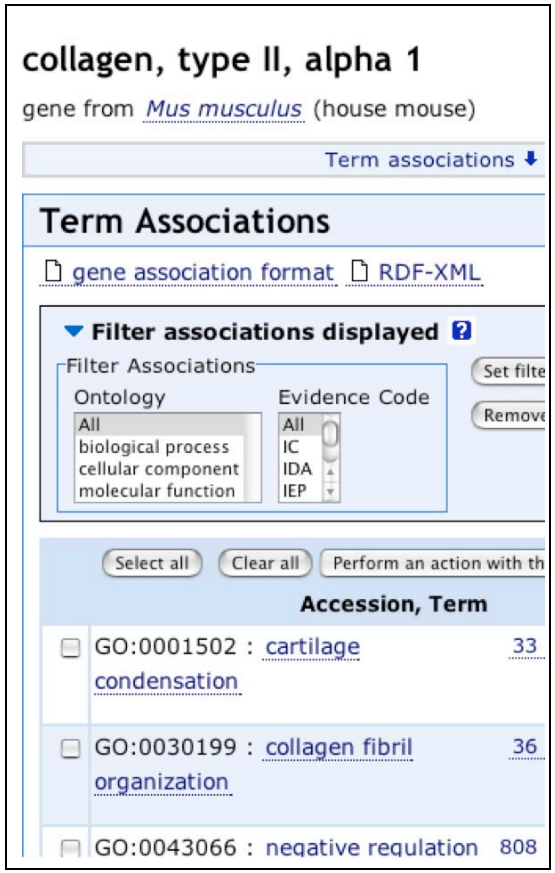
See D. Endy, *Nature* **433**: 449

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
  - avoid reinventing the wheel
  - integration of information across levels
- Examples
  - MIAME for microarrays
  - Gene Ontology (protein functions)
- Who drives standards?
  - scientists, funding agencies, journals, industry



http://www.geneontology.org/
Screenshot image captured April 2010.
Courtesy of the Gene Ontology.
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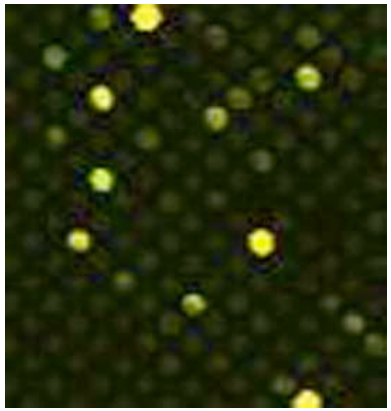
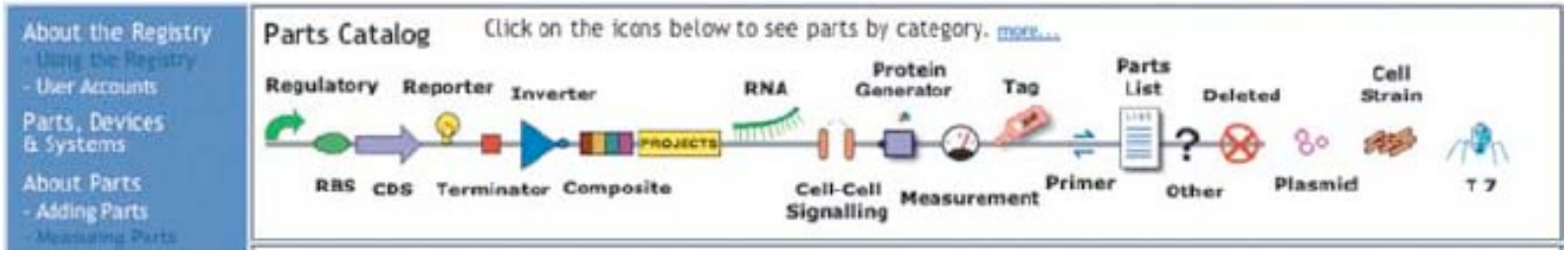
Lecture 3: conclusions

- Confidence intervals and t-tests are two useful statistical concepts.
- Standardizing data sharing and collection is of interest in several BE disciplines.

Microarray data



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



Next time: *discussion* of standards in TE; more about cell viability and microscopy

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