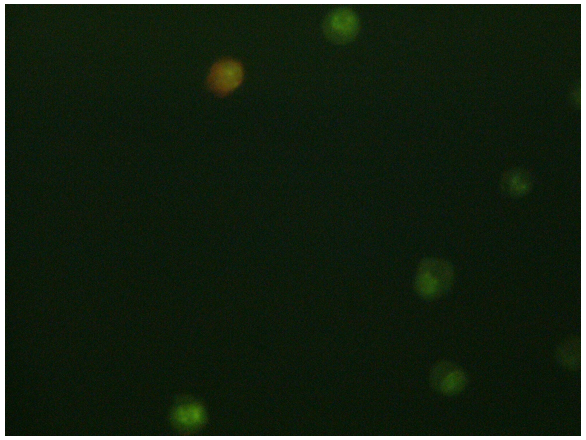
Cell viability in TE constructs

Module 3, Lecture 4

20.109 Spring 2009

Lecture 3 review

- What engineering principles may be useful food-for-thought in BE?
- What difference in live and dead cells is exploited in the Day 3 assay?

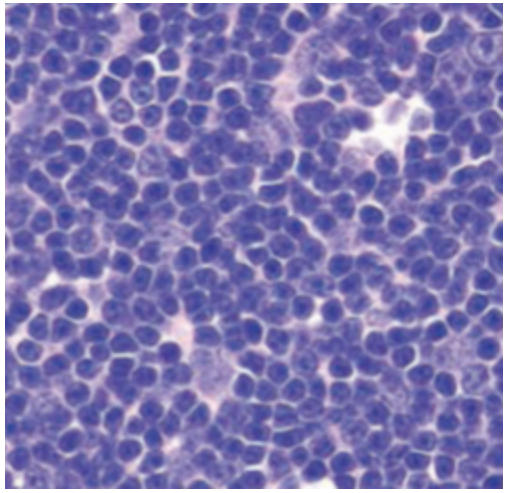


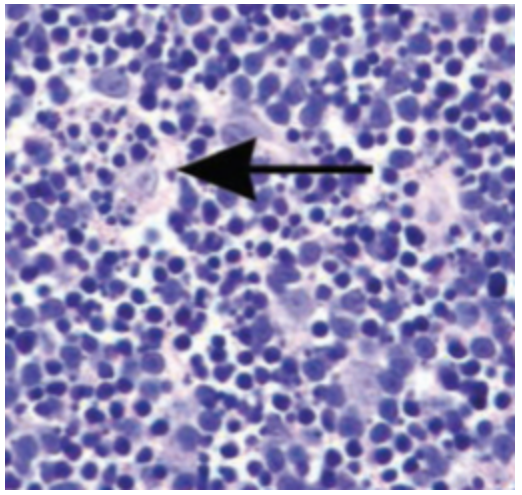
Topics for Lecture 4

- Cell viability
  - background
  - relation to diffusion
  - your data
- Fluorescence microscopy
- Overview: remainder of Module 3

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
- Distinguish by morphology or biochemistry

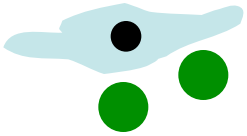


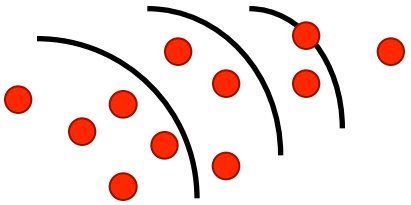


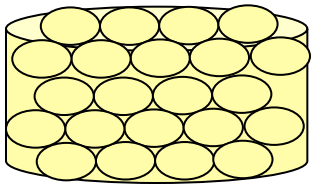
Images: S. Elmore *Toxicol Pathol* **35**:495 (2007)

Factors affecting cell viability

- Cell-related
  - density
  - interactions
- Cytokine-related
  - promote viability or proliferation
  - promote apoptosis
- Materials-related
  - bulk permeability
  - pore size, percent porosity
  - toxicity

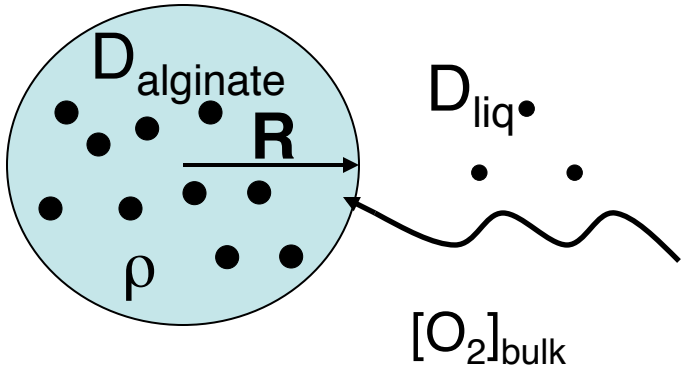


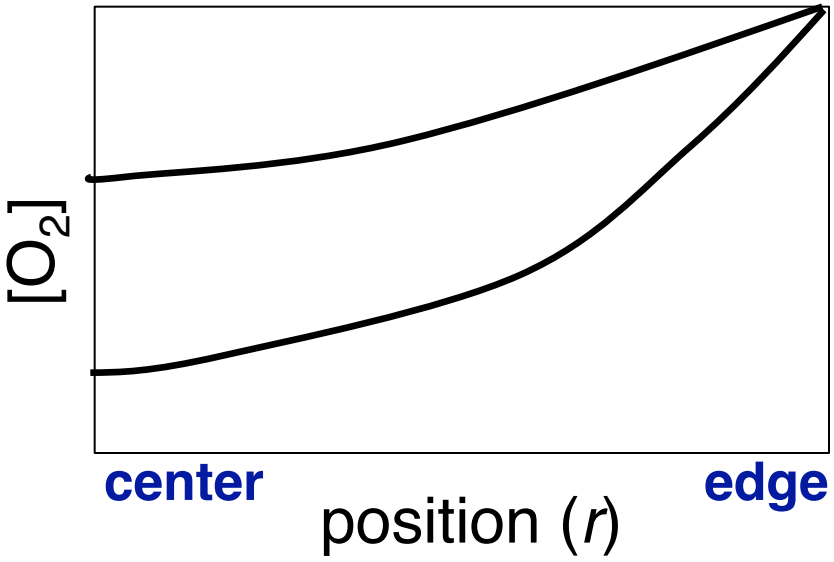




Nutrient use in 3D constructs

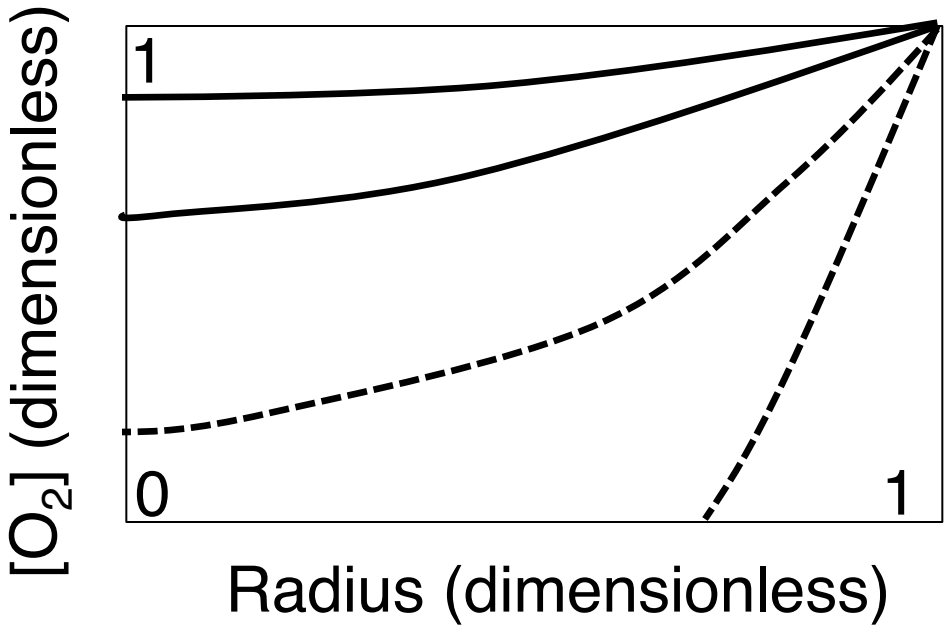
- Relevant parameters
  - size of construct R
  - cell density ρ
  - diffusivity D
  - conc. in medium $[O_2]_{bulk}$
- Concentration profile
  - can be solved
  - [O2] ↓ toward center
  - steepness depends on above parameters





Diffusion limits

- Characteristic diffusion limit (nutrients, O_2): ~100 μm
- Diffusion and viability profiles correlated
- Solution *in vitro*: dynamic/perfusion culture
- Solution *in vivo*: promote angiogenesis quickly



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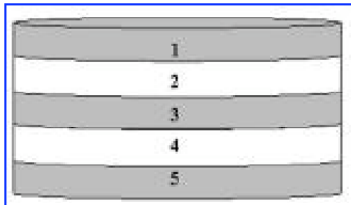
Diffusion- limited regime (high consumption)

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

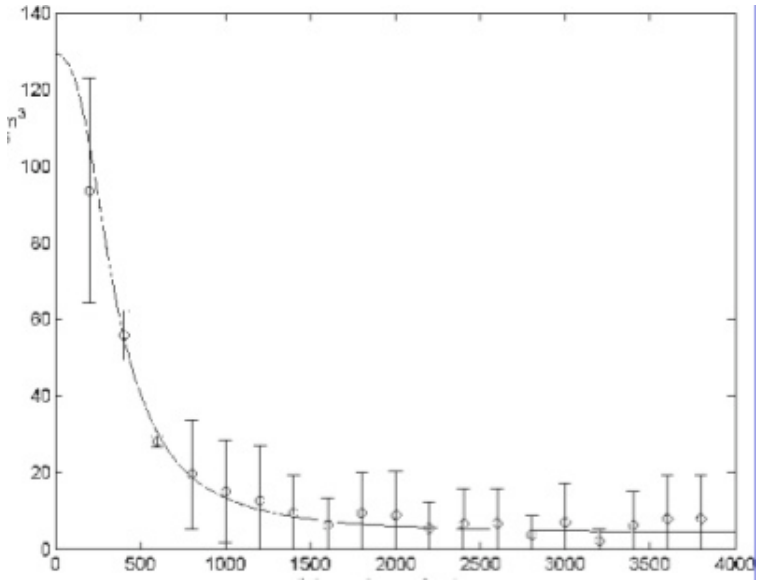


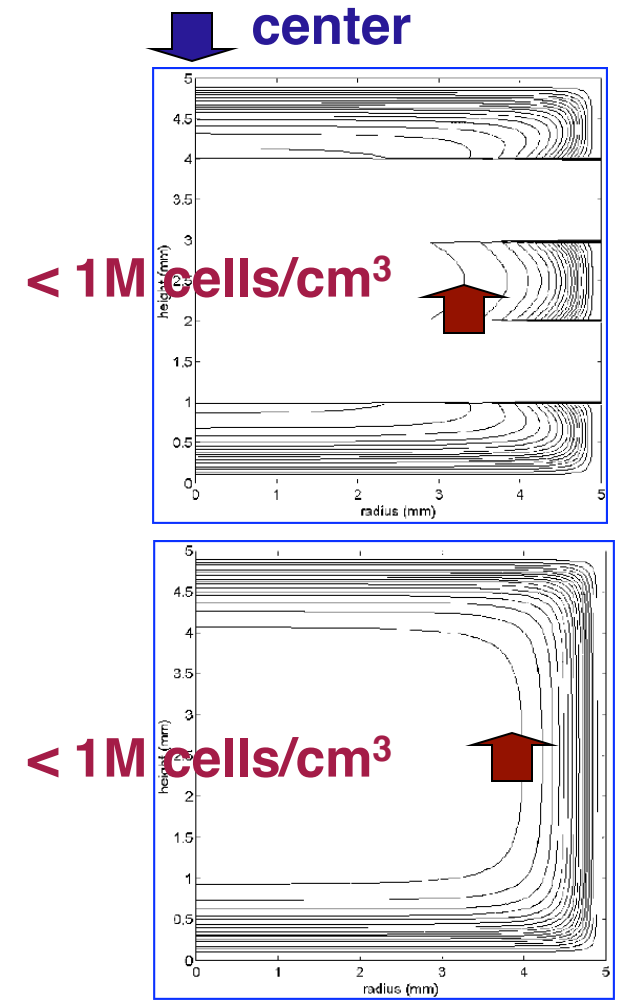
B Cells in all layers

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

Dunn et al. results for cell viability

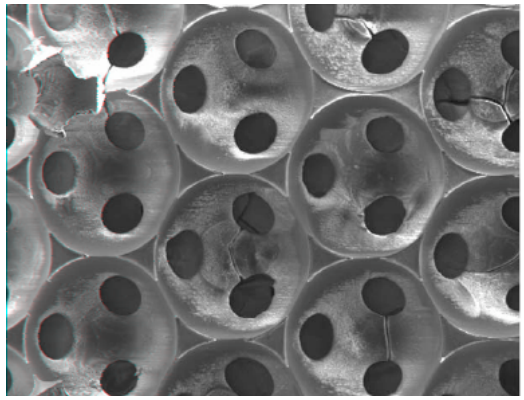
- A more uniform than B
- Cell growth matches O_2 tension
- Claim of predictive capability





Modeling diffusion in a defined porosity

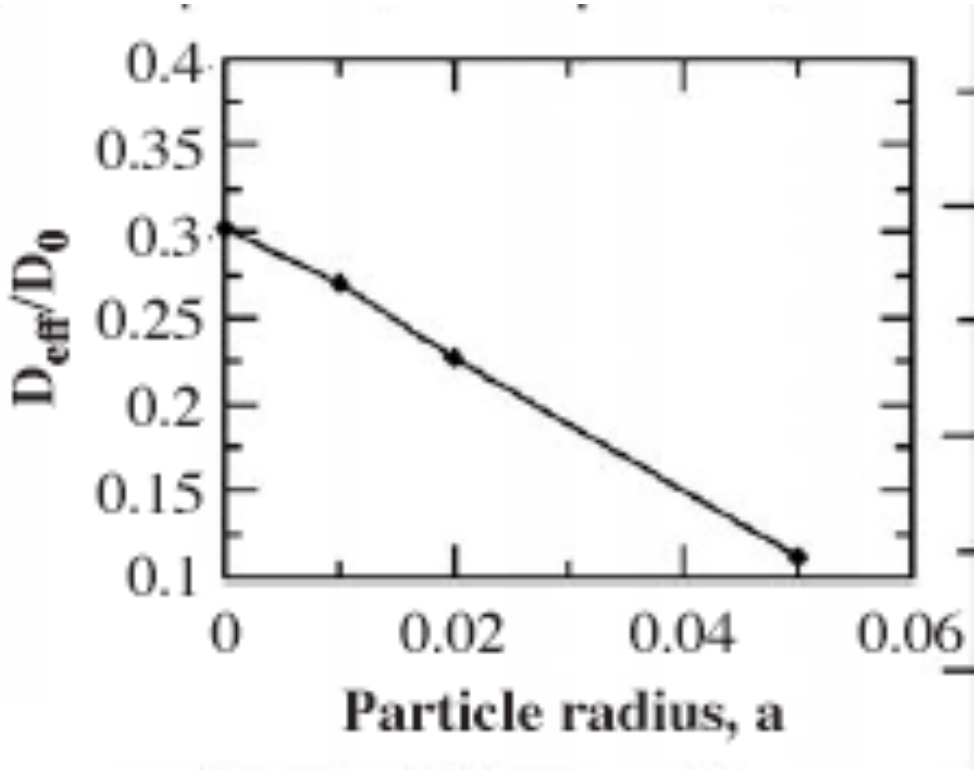
- Geometrically defined space
- Simulate particle motion
- Brownian dynamics
  - Idealized point particles
  - Excluded volume (can't cross wall)
  - Equations of motion
- Monte Carlo
  - Finite particles
  - Excluded volume (wall *and* particle)
  - Boltzmann probabilities to evaluate steps



S. Shanbhag et al. *Biomaterials* **26**:5581(2006)

Results: diffusion in a defined porosity

- Upper bound: $D_{scaffold} = 0.3\ D_{soln}$
- Decreases with inter-pore size
- … w/particle size
- … w/ confinement
- … w/ consumption



Interlude: accessibility, audience

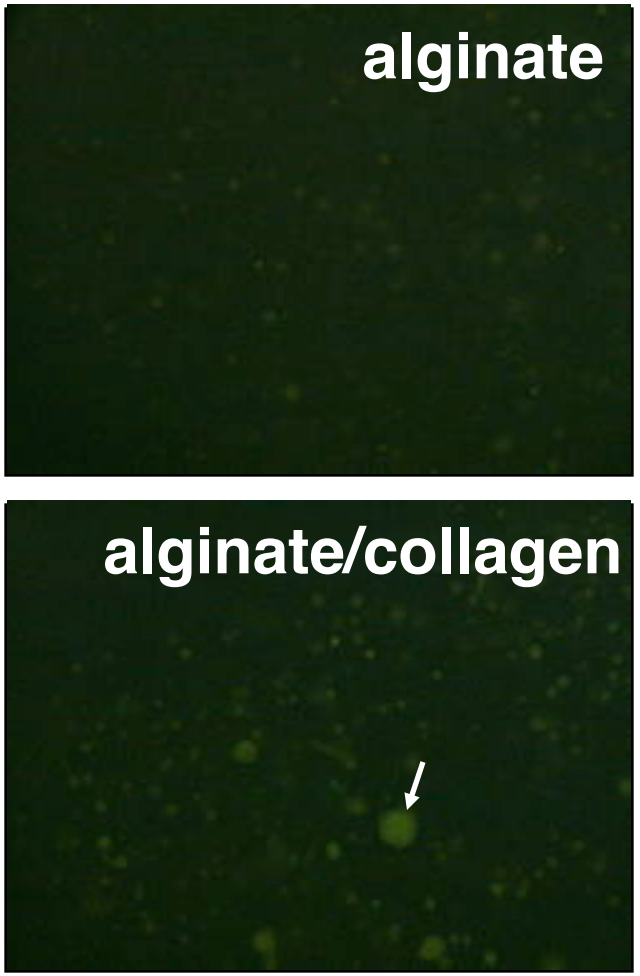
1. Slings and Arrows
   S1E3, corporate seminar

2. Publishing and the petabyte age
   Letter re: publishing
   http://www.nature.com/nature/journal/v455/n7209/full/455026a.html
   Petabyte age issue
   http://www.nature.com/nature/journal/v455/n7209/full/455001a.html

Module progress: week 2

- Day 3: viability/cytotoxicity testing
- Practical matters
  - focusing takes practice
  - signal:noise
- Groups generally found
  - mostly live
  - mostly round
  - some clustering
- How can we explain these results?
- How can we improve the assay?
- Possible trends

W/F Purple group



Fluorescence microscopy

- Light source
  - Epifluorescence: mercury, xenon
  - Confocal: laser (Ar, HeNe)
  - 2-photon: pulsed laser
- Filter cube
  - Excitation
  - Dichroic mirror
  - Emission
  - Band-pass vs. long-pass
- Detection
  - CCD camera

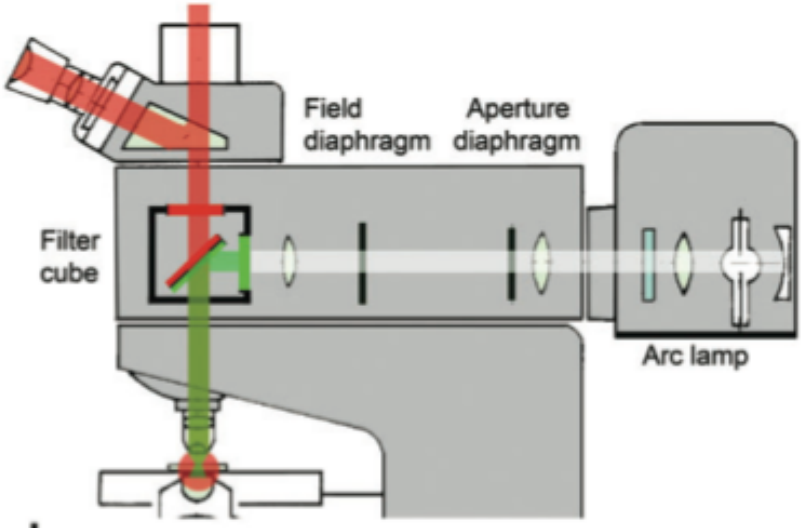
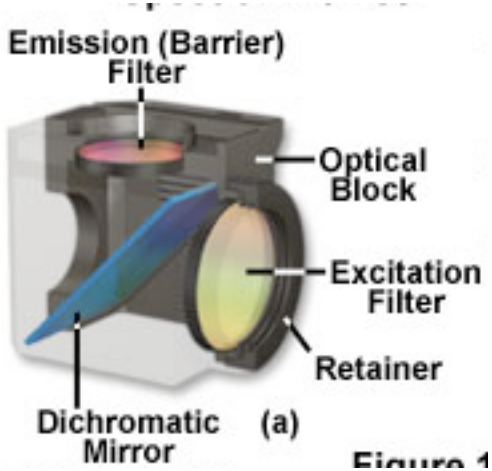
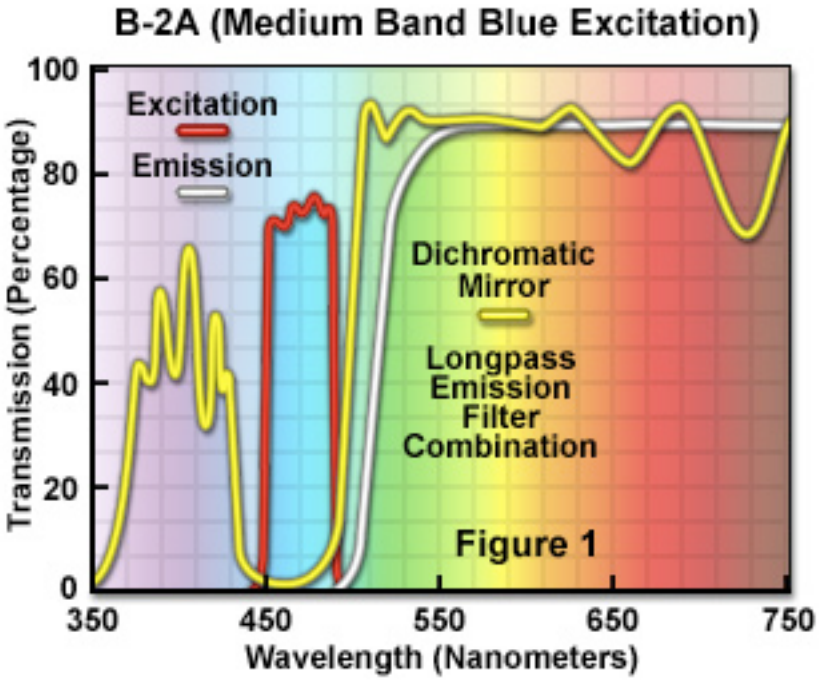


Image from: Lichtman & Conchello, *Nature Methods* **2**:910 (2005)

Specifications for Day 3 imaging

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

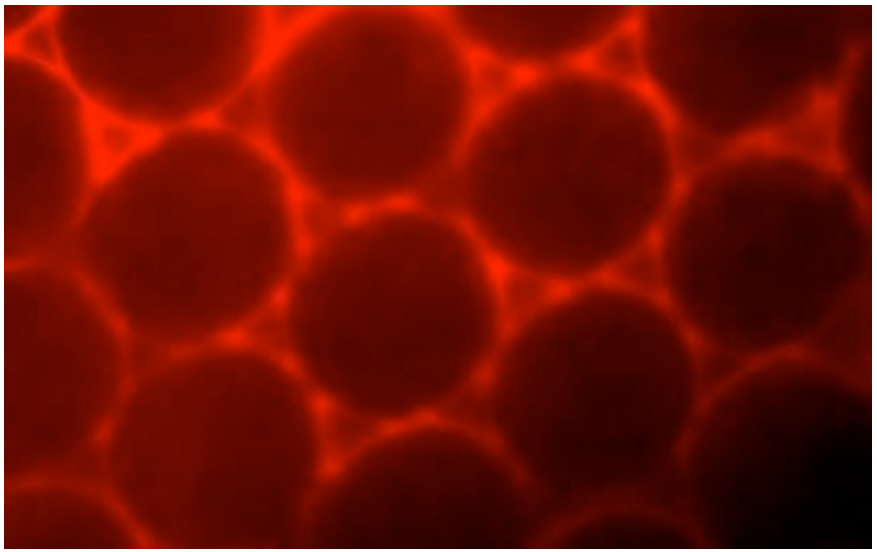




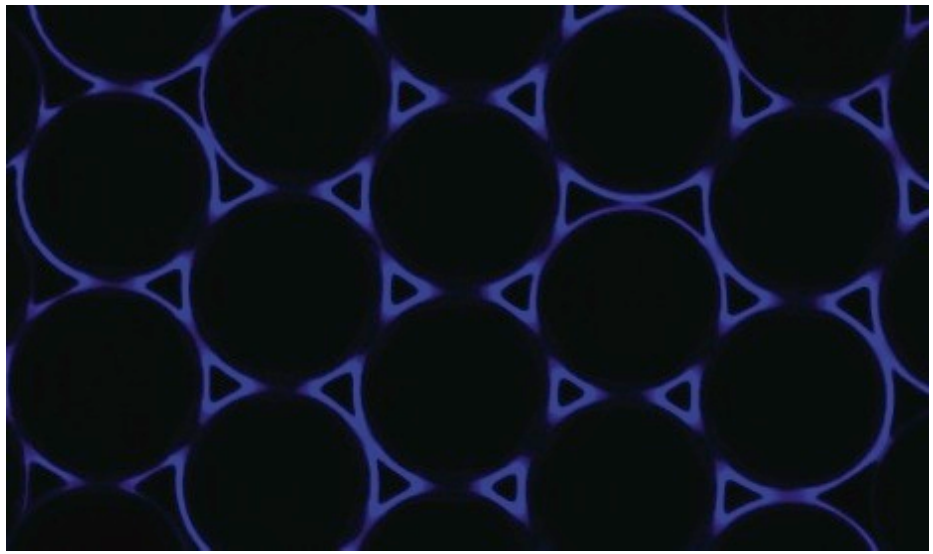
Images from: Nikon microscopy website: www.microscopyu.com

Types of microscopy

- Epifluorescence: out-of-plane light makes noise
- Confocal: pinhole rids out-of-plane light
- 2-photon: femtoliter volume excited

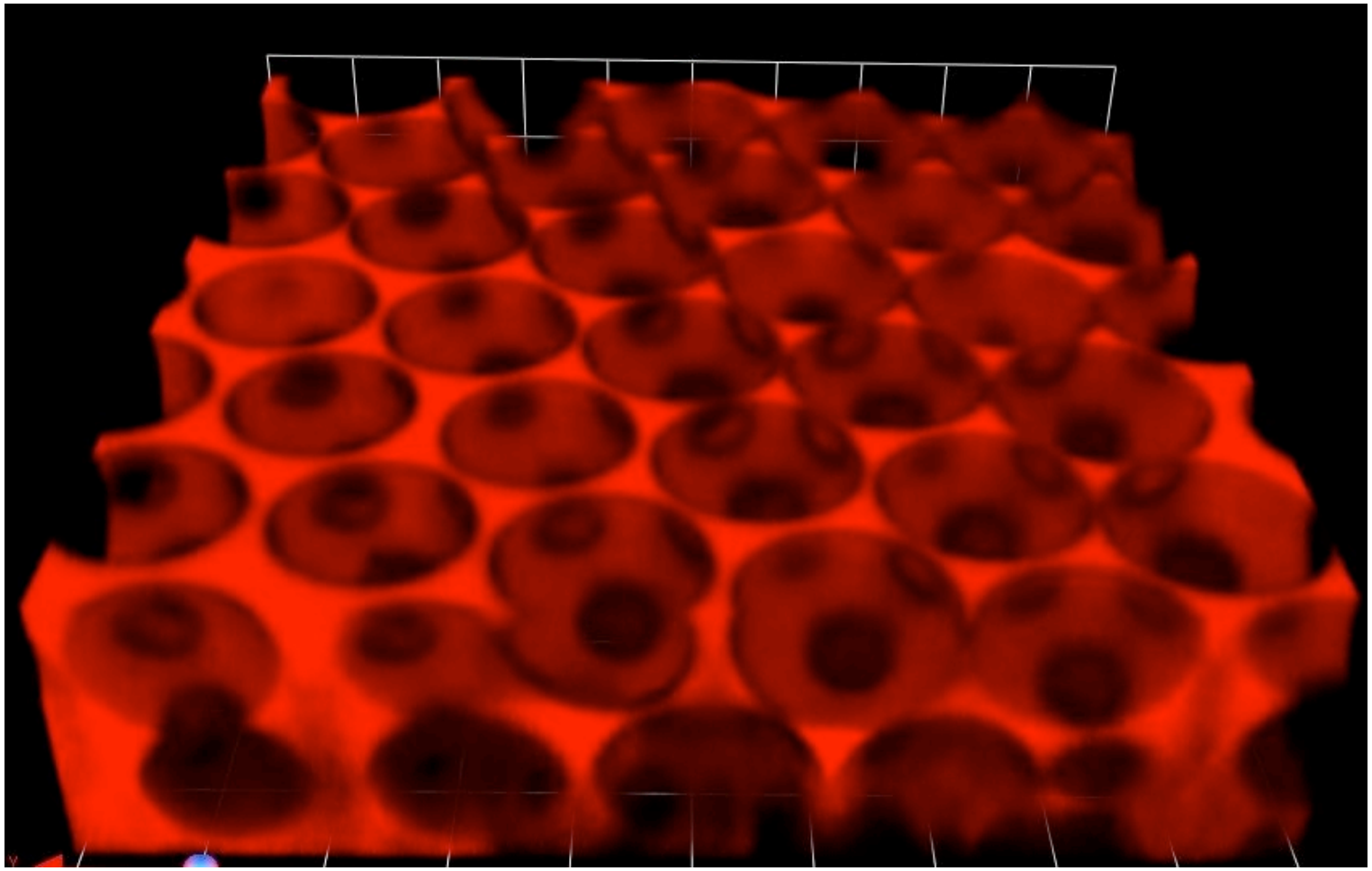


Epifluorescence



Confocal

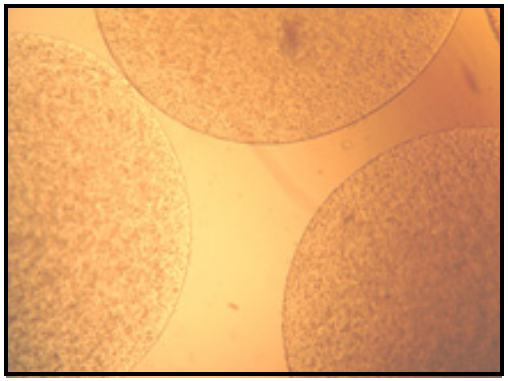
Confocal uscopy permits 3D reconstruction



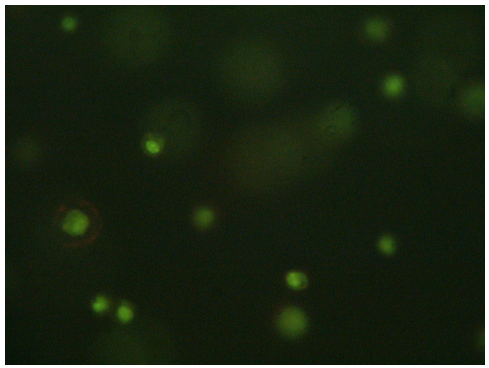
Module overview

Day 1: design

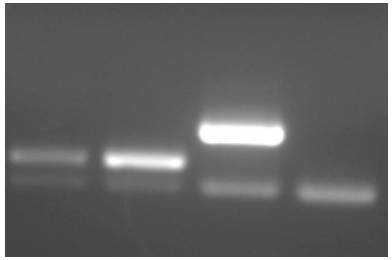
Day 2: seed cultures



Day 3: viability assay



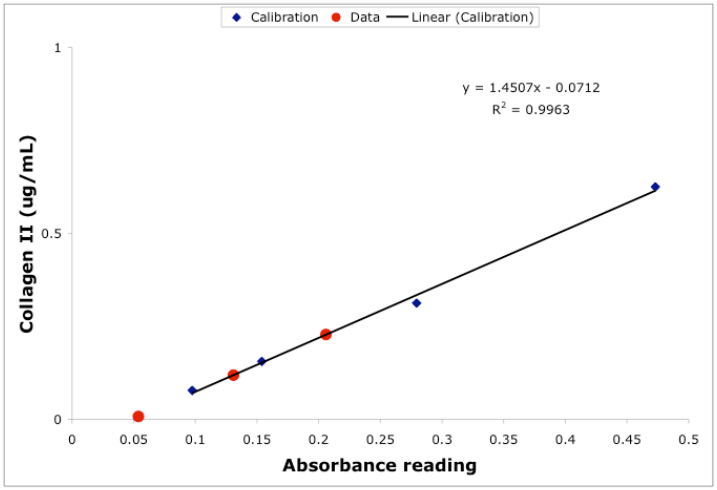
Day 4: prep RNA+cDNA



Day 5: transcript assay

Day 6: protein assay

Day 7: remaining analysis



Day 8: your research ideas!

Lecture 4: conclusions

- Cell viability in TE constructs is affected by factors at the cell, materials, and cytokine level.
- Modeling is one useful tool to study the effects of nutrient diffusion on cell viability.
- Fluorescence imaging can be exploited to study both cells and scaffolds.

Next time: transcript and protein assays, *in vitro* and *in vivo* models for cartilage TE