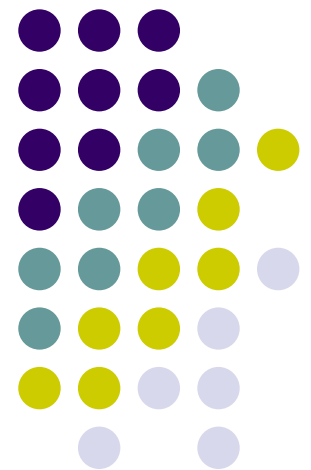
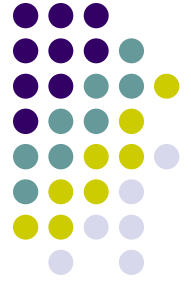


HDF Cell Proliferation and Viability

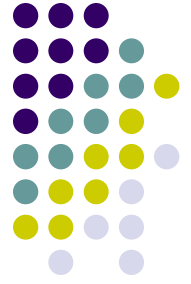
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Objectives

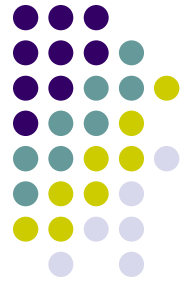


- Establish relationship between absorbance and concentration of viable cells
- Examine the number of cells entering mitosis at a given point in time
- Evaluate the rate of proliferation under different media conditions



Measuring Cell Viability

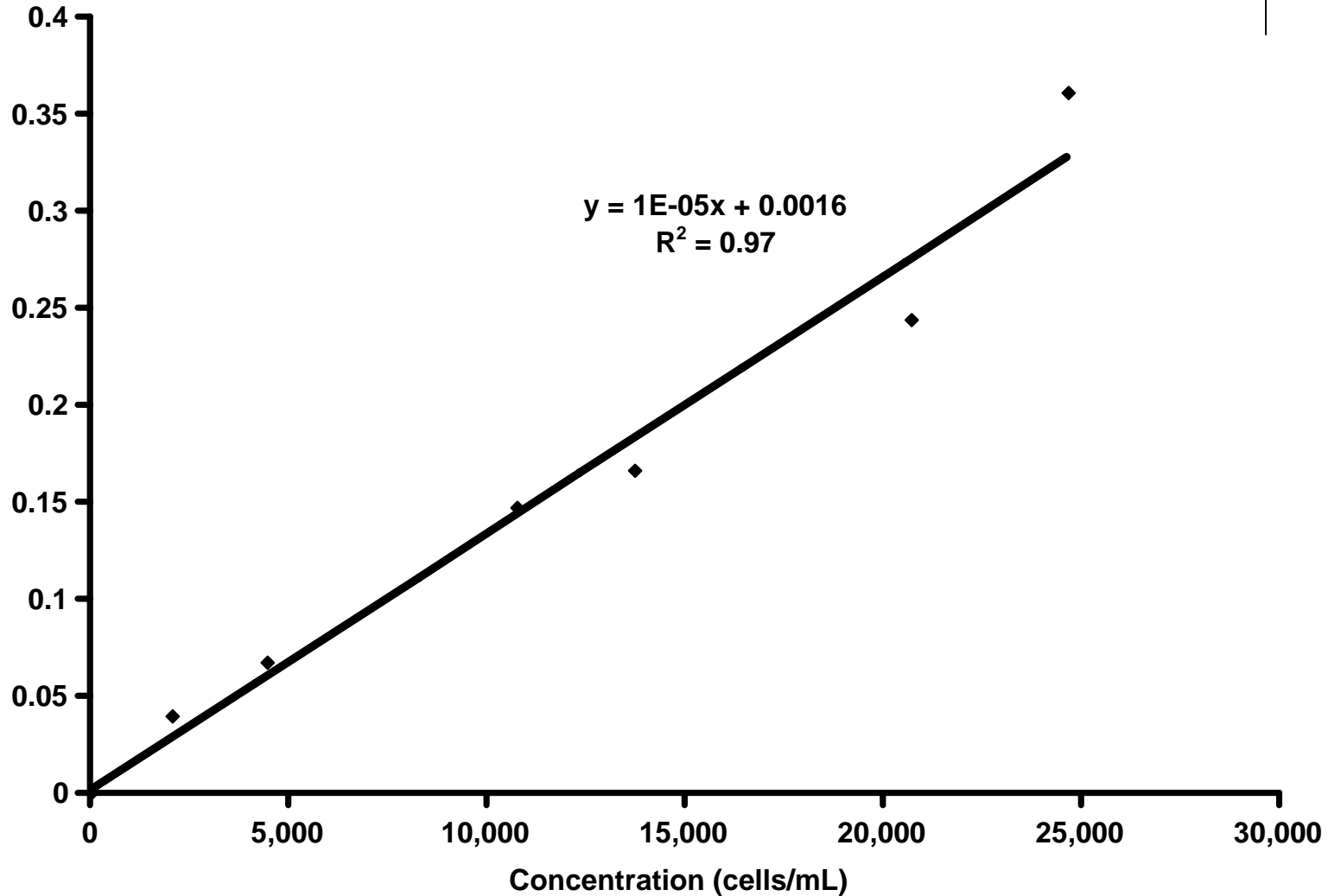
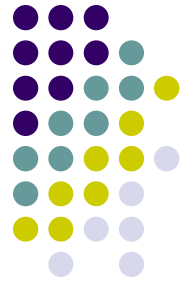
- MTT Viability Assay
 - Seed cells in 10% FBS media in 2 sets of 7 dilutions
 - Incubate cells for 2 days
 - Count cells in one set of dilutions with the coulter counter (diameter $\geq 10.5\mu\text{m}$)
 - Treat cells in second set of dilutions with MTT
 - Use spectrophotometer to measure absorbance



Proliferation Measurements

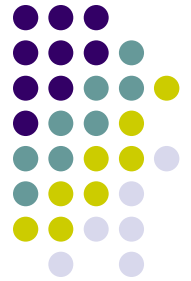
- Cell Proliferation Assay
 - Seed 5,000 cells in each well on day 0 in 10%, 5%, and 1% FBS media
 - Count cells on day 0, 2, 5, and 7 using a coulter counter (diameter $\geq 10.5\mu\text{m}$)
- Anti-PCNA Assay
 - Seed 20,000 cells in each well on day 1 in 10%, 5% and 1% FBS media along with 3 controls
 - Incubate for 2 days
 - Treat cells with Anti-PCNA then secondary antibody
 - Inspect cells under light microscope

Linear Relationship Exists for Absorbance and Concentration



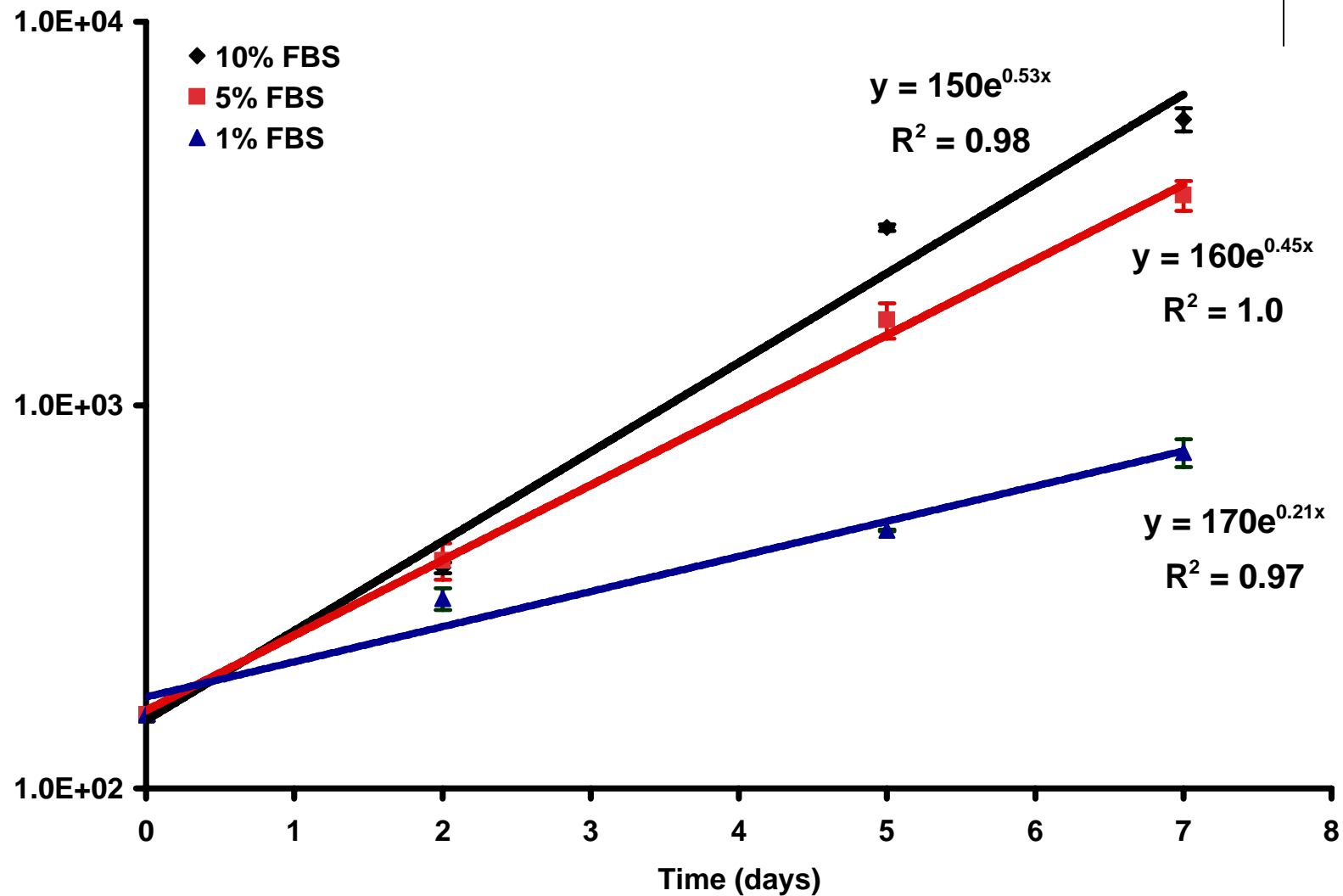
*Data from XXXX

Linear Relationship Allows for Counting of Viable Cells

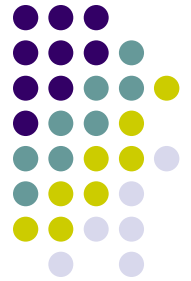


- Live cells metabolize MTT
 - Turns yellow dye purple
- Larger amount of cells results in more metabolism
- Using linear relationship and measuring absorbance, concentration of viable cells is approximated

Growth of Cells Is Exponential



Proliferation Rate Is Dependent on FBS Concentration

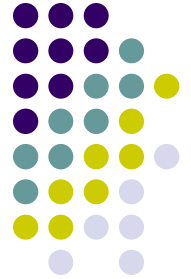


Sample	Doubling Time
10% FBS	1.3 days
5% FBS	1.5 days
1% FBS	3.3 days

- Cell count on day 7 found to be highest for 10% and lowest for 1% FBS*
- Cell count increases between day 2, 5, and 7 for 10% FBS media*

*ANOVA $p < 0.001$ and Tukey HSD $p < 0.05$

Serum Concentration Affects Number of Cells in Mitosis



Sample	Percent of Cells in S Phase
10% FBS	80% Cells
5% FBS	60% Cells
1% FBS	10% Cells

- Cell nuclei stain red in S phase when PCNA is produced
- Cell nuclei stain blue under other conditions
- All control groups stained blue showing only specific binding of primary and secondary antibodies

Cell Proliferation Depends on Number of Cells in Mitosis



- Anti-PCNA and cell proliferation assays display 10% FBS media proliferates cells better than 5% and 1% FBS
 - Anti-PCNA shows greatest percentage of cells in S phase after 2 days incubation for 10% FBS
 - Doubling time for 10% FBS is the shortest from proliferation assay
- More mitotic cells correlates to a greater proliferation rate

Summary of Proliferation and Viability



- Linear relationship exists between absorbance and viable cell concentration
 - Allows for counting only viable cells using calibration curve and absorbance measurements
- Anti-PCNA shows greater percentage of cells in S phase for 10% FBS media
- 10% FBS media grows cells faster than 5% and 1% FBS
 - Shorter doubling time and more cells present after 7 days