Lecture 14:
Quantifying Cell Function

c. Cell Proliferation Assays

Importance:

- wound healing
- immune host defense
- cancer therapy
- tissue engineering
- bioprocessing/synthesis of theraputic glycoproteins

➢ *Cell number*

1. count via microscopic observation or Coulter counter

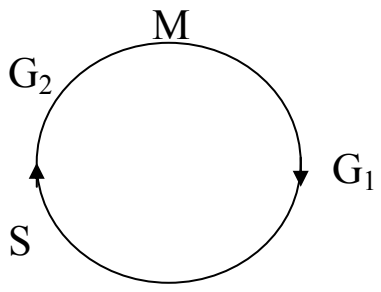
2. compute specific *cell proliferation rate const*:

$$k_g = \frac{1}{N}\frac{dN}{dt} = \frac{d(\ln N)}{dt} \qquad \text{(units: } t^{-1}\text{)}$$

N = # of cells at time t

➢ *Cell phase populations*

In some cases, we want to know the population of cells in each phase of the cell cycle (eukaryotes):



M = Mitosis (~ 1 h)

G_1 = gap between cell division & DNA synthesis (~18-72 h)

S = DNA synthesis (~6-8 h)

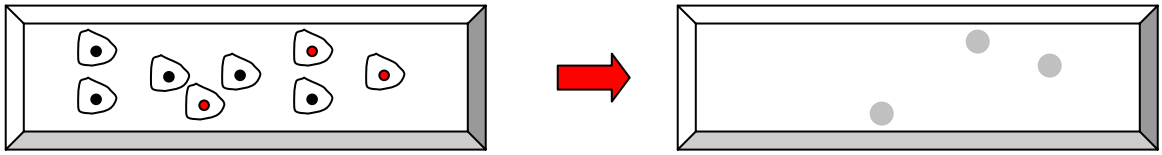
G_2 = gap between DNA synthesis & mitosis (~2-3 h)

G_o = quiescent cells

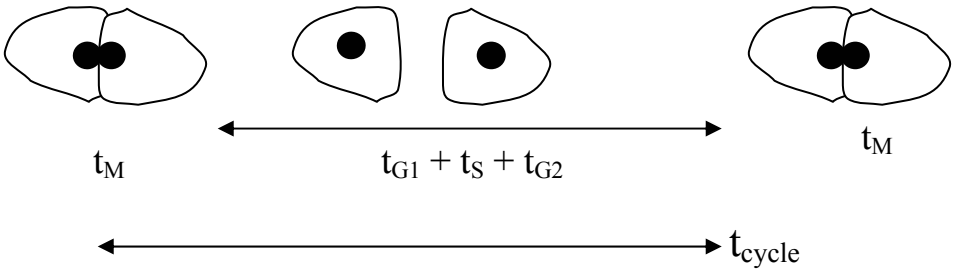
tritiated-thymidine uptake

1. cells exposed to [^{3}H]thymidine pulse which labels S-phase cells only

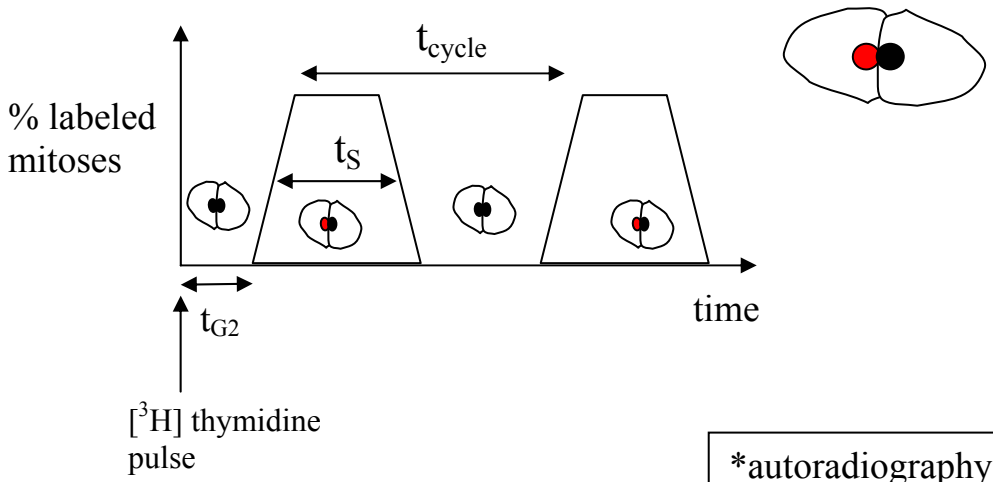
2. % S obtained from *autoradiography* (Ag precipitates in an overlying emulsion film reveal S-phase cells, similar to a photograph emulsion*)



3. % M and t_M obtained from optical microscopy (visually distinct)



4. by following % labeled mitoses after [^{3}H]thymidine pulse through 2 cell divisions, t_{G2}, t_S and t_{cycle} can be determined → t_{G1}



*autoradiography is also used with PAGE for protein identification

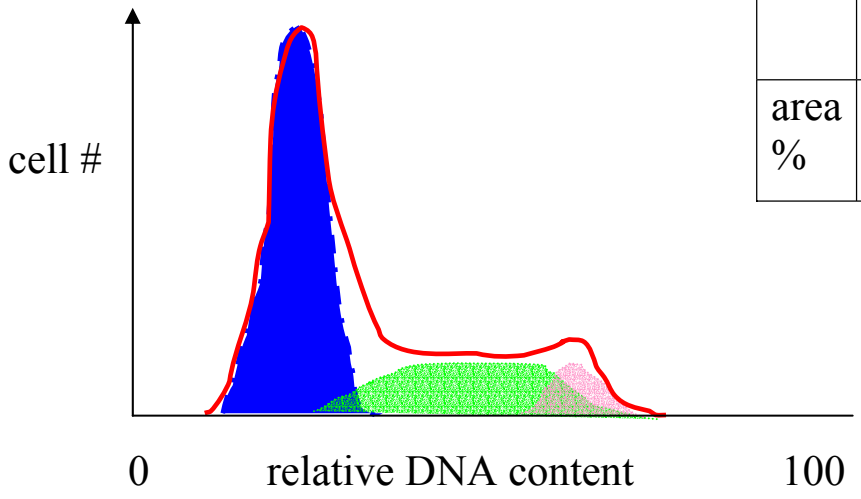
*flow cytometry**

1. cells' DNA labeled with DNA-specific fluorescent dye (ex. acriflavine, ethidium bromide)

2. count cell # vs. fluorescence using laser excitation

3. fluorescence intensity ~ DNA present

G_1:S:G_2:M DNA (& fluorescence) ratio is 1:1-2:2:2



	G_1	S	G_2+M
area %	52	41	7

Advantages:

- no radiolabeling
- faster than autoradiography

Drawback:

- M & G_2 populations lumped

*flow cytometry is also used to determine expression of specific cell receptors by labeling with fluorescent antibodies

d. Cell Differentiation Assays

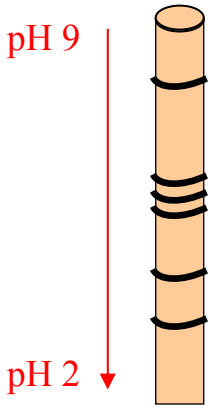
Importance:
- change in phenotype renders specialization of function
- characterized by changes in protein synthesis, genes expressed & secretions

2D Gel Electrophoresis (SDS-PAGE): cell proteins separated on basis of mass and charge—providing a "signature" of the cell

Method

1. cell is lysed (contents extracted)

2. protein solution is separated by pI (isoelectric point = pH where net charge = 0) using *isoelectric focusing*



i. ampholyte mixture is placed under E-field to create a stable pH gradient in a polyacrylamide gel.

ii. protein solution is added and E-field reapplied

iii. proteins diffuse to their isoelectric point

3. gel strip is next placed crosswise on a second gel incorporating sodium dodecyl sulfate (SDS)

4. SDS binds & denatures proteins ⇒ native charge becomes negligible
~1 SDS per 2 amino acids

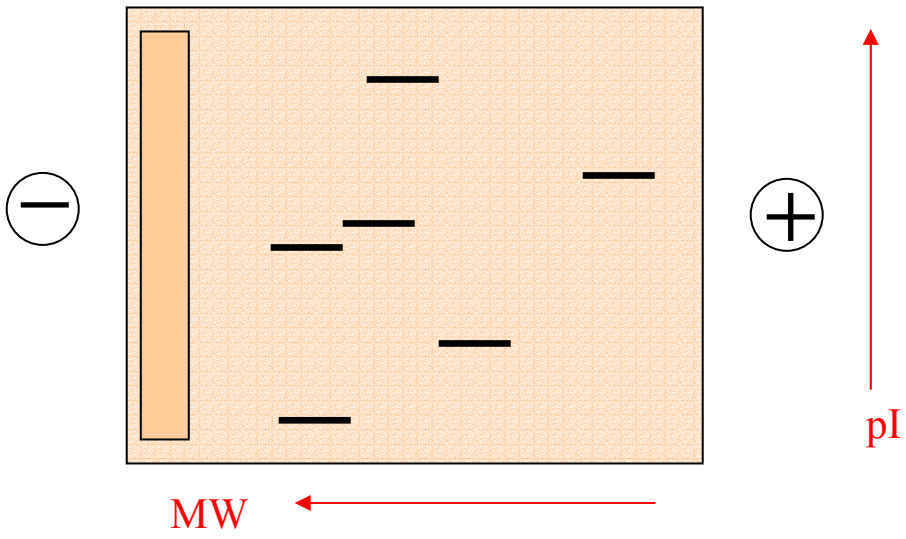
charge/mass ≈ constant

5. gel is placed under E-field, separating protein chains via molecular weight

low molecular weight species move faster

$$\mu = \mu(c_{gel}, E, MW_{protein})$$

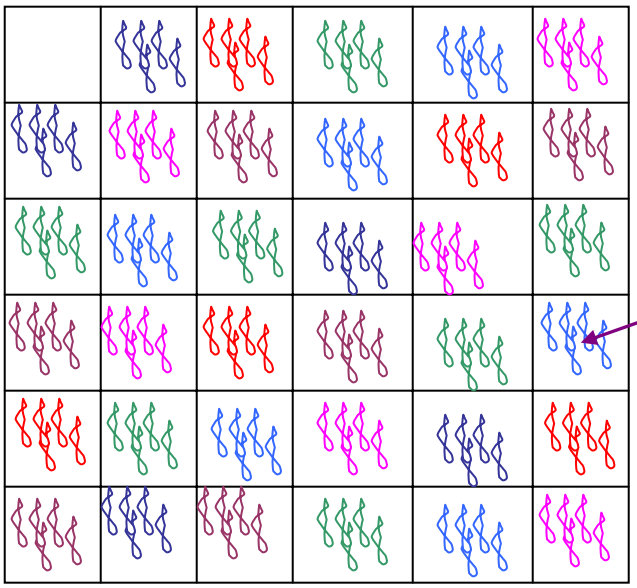
SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis



6. compare “before” and “after” signature to discern differentiation

DNA microarrays: test for gene expression
(first commercialized by Affymetrix)

array of "probes" comprising surface-bound gene fragments
(~20 bases/fragment)



- 2M probes/chip
- up to 40,000 genes/chip

~50 μm^2 probe area =
10M DNA fragments

Method

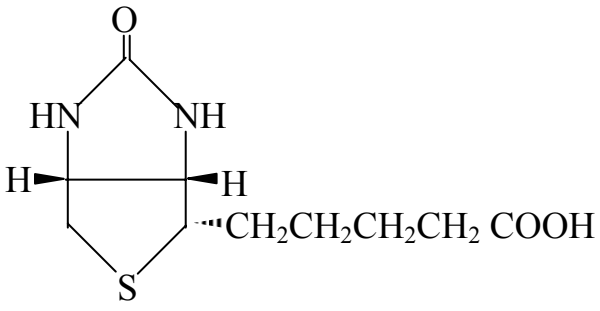
1. cells lysed to retrieve mRNA

2. mRNA transcribed to cDNA (complement), which is then transcribed to cRNA

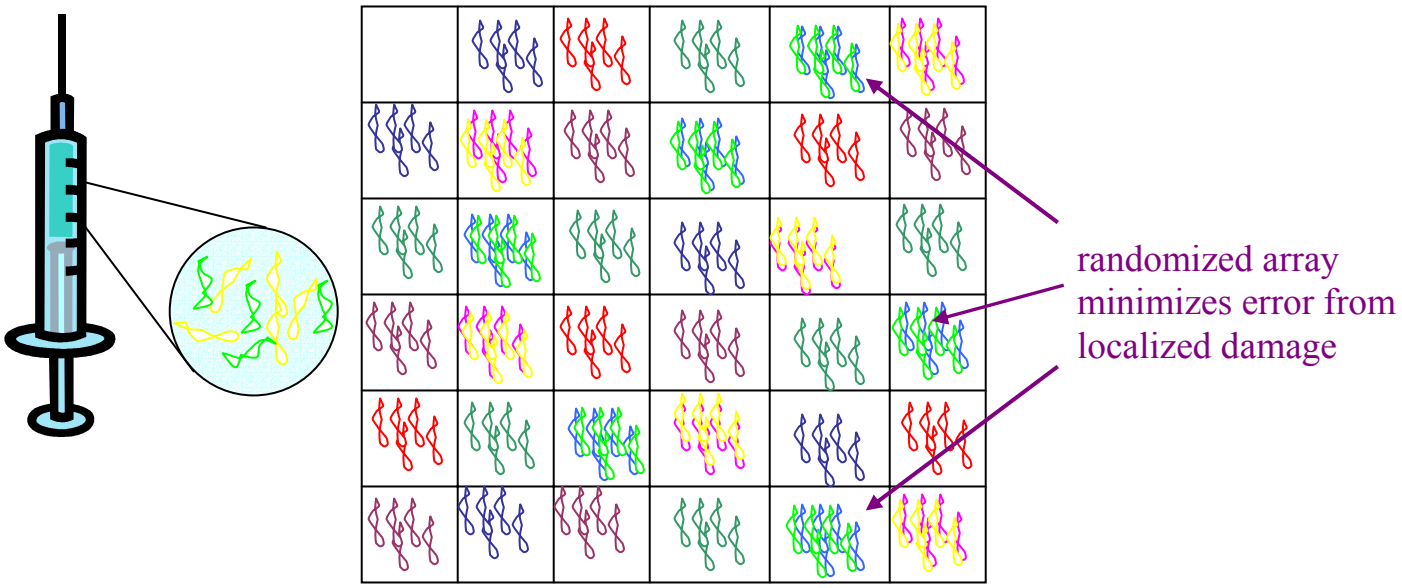
3. cRNA is cut into 35-200 base fragments & labeled with biotin (vitamin H)—referred to as "biotinylation"

Biotin has strong binding affinity for streptavidin

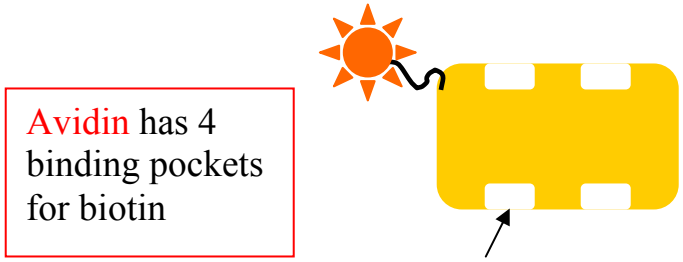
$K_a \sim 10^{15}$ L/mol



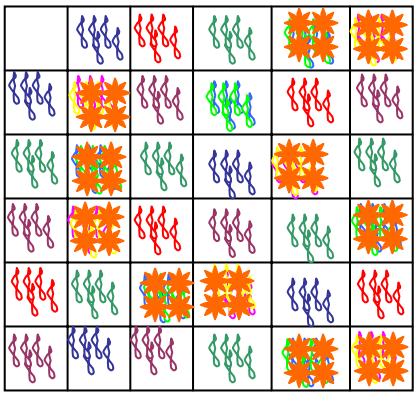
4. cRNA "cocktail" is added to array, hybridizes with DNA fragments



5. surface-bound cRNA-biotin is labeled with fluorophore-labeled avidin

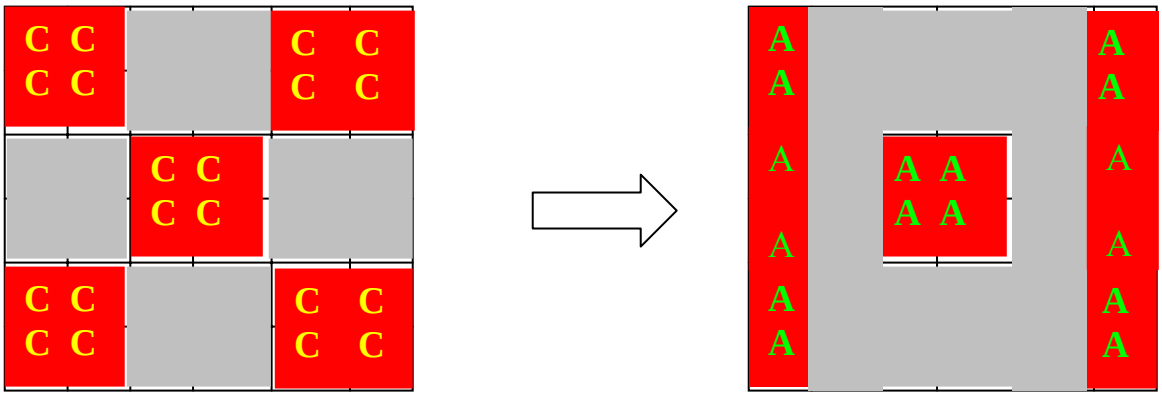


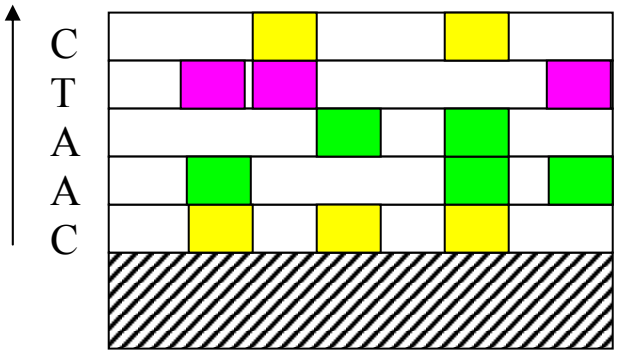
6. fluorescence spatial readout determines genes being expressed



DNA Array Fabrication

Lithographic masks used to build up A-C-T-G sequences base-by-base





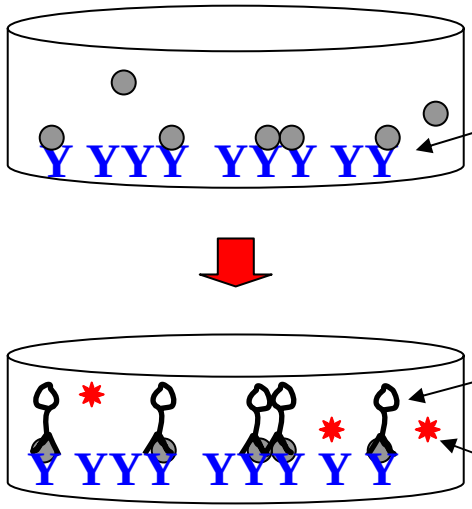
S.P.A. Fodor et al., *Science* **251**, 767 (1991).

e. Secretion

Enzyme-linked immunosorbent assay (ELISA): tests for a particular protein's presence in cell secretions

1. cell secretions aspirated from culture medium

MAb surface made by direct adsorption or protein adsorption followed by MAb



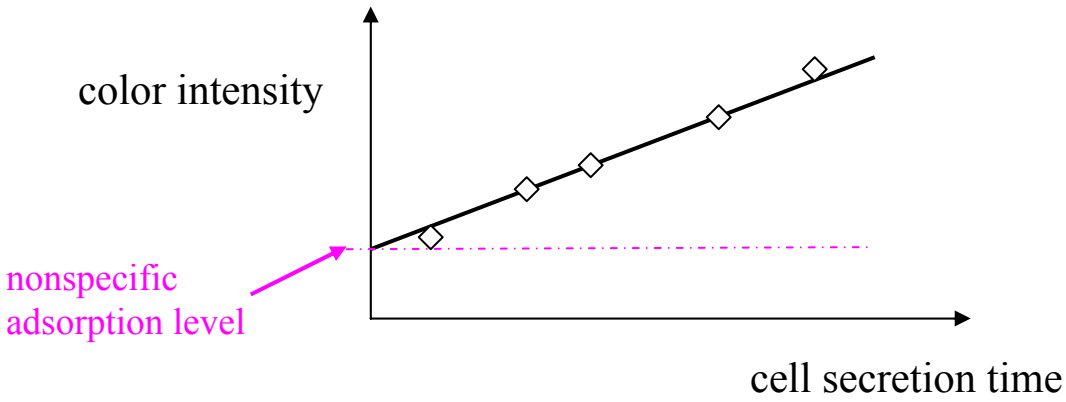
2. surface coated with soln of monoclonal antibody (MAb) for protein of interest—protein binds MAb if present

3. 2nd antibody (enzyme-linked) is added—binds to protein on surface to create a "sandwich"

4. The enzyme (ex., alkaline phosphatase) catalyzes conversion of a compound to a colored product (amplification)

4. Color intensity is read in spectrophotometer

5. Nonspecific adsorption is determined and subtracted



Alternately, proteins directly adsorbed on a surface may be probed by ELISA in which the second (enzyme-linked) antibody is an antibody to the first antibody.