Cell, Tissue, and Tumor Kinetics (Chap. 21)

To study the effect of Tumor & Tissue Kinetics on TCP & NTCP

The Study of Cell Cycle

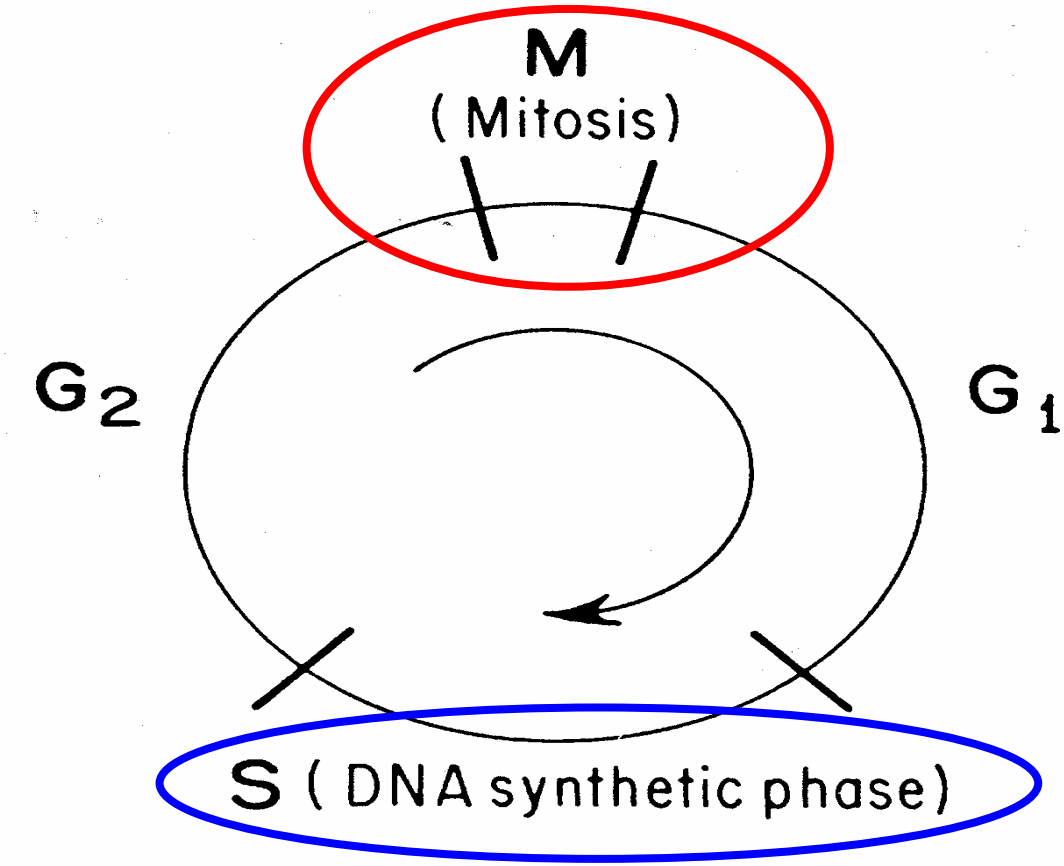
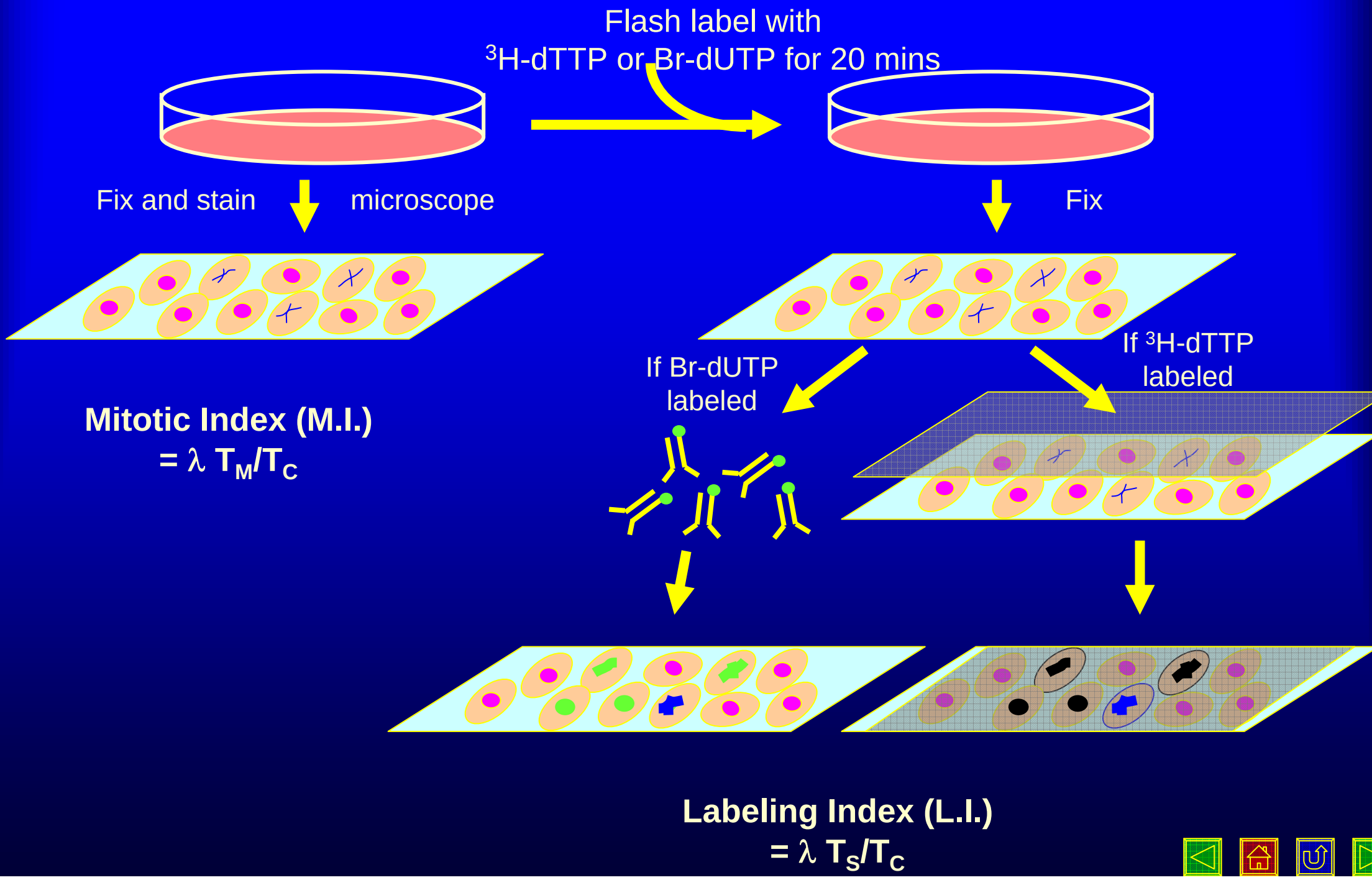


Figure 21.1. The phases of the cell cycle. Mitosis (M) is the only event that can be distinguished through the light microscope. The DNA synthetic phase (S) may be identified by the technique of autoradiography (Chapter 4). The intervals of apparent inactivity are labeled G_1 and G_2.

Cell Cycle

- Techniques for measuring cell cycle parameters most often use thymidine analogues that are taken up in S (DNA synthesis) phase
  - Tritiated thymidine (autoradiography).
  - Bromodeoxyuridine (fluorescent antibody)

Methods for studying Cell cycle



Mitotic Index (M.I.)
$= \lambda\ T_M/T_C$

Labeling Index (L.I.)
$= \lambda\ T_S/T_C$

Measurement of Cell Cycle Parameters

1. Cell cycle time (Tc)
2. Mitotic Index (MI) => Tm
3. Labeling Index (LI) => Ts
4. Percent Labeled Mitosis (PLM) =>

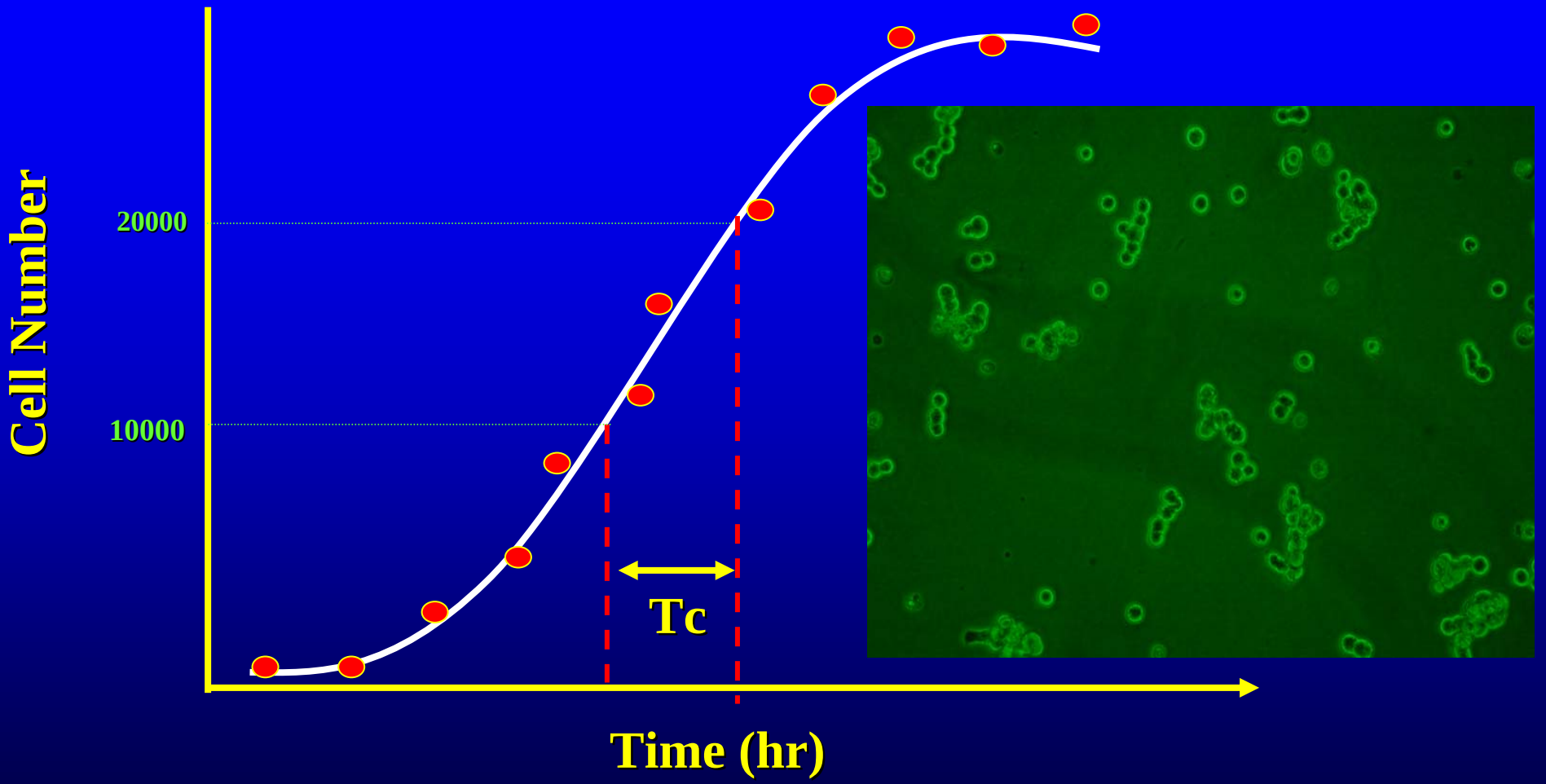
   Tc, Tm, Ts, T_{G1}, T_{G2}

5. FACS 

Cell Cycle Time (Tc)

- **Cell Growth Curve (Counting cell number)**
- **Percent labeled mitosis**
- **FACS**

Cell Cycle Time (Tc)



Mitotic Index (MI)

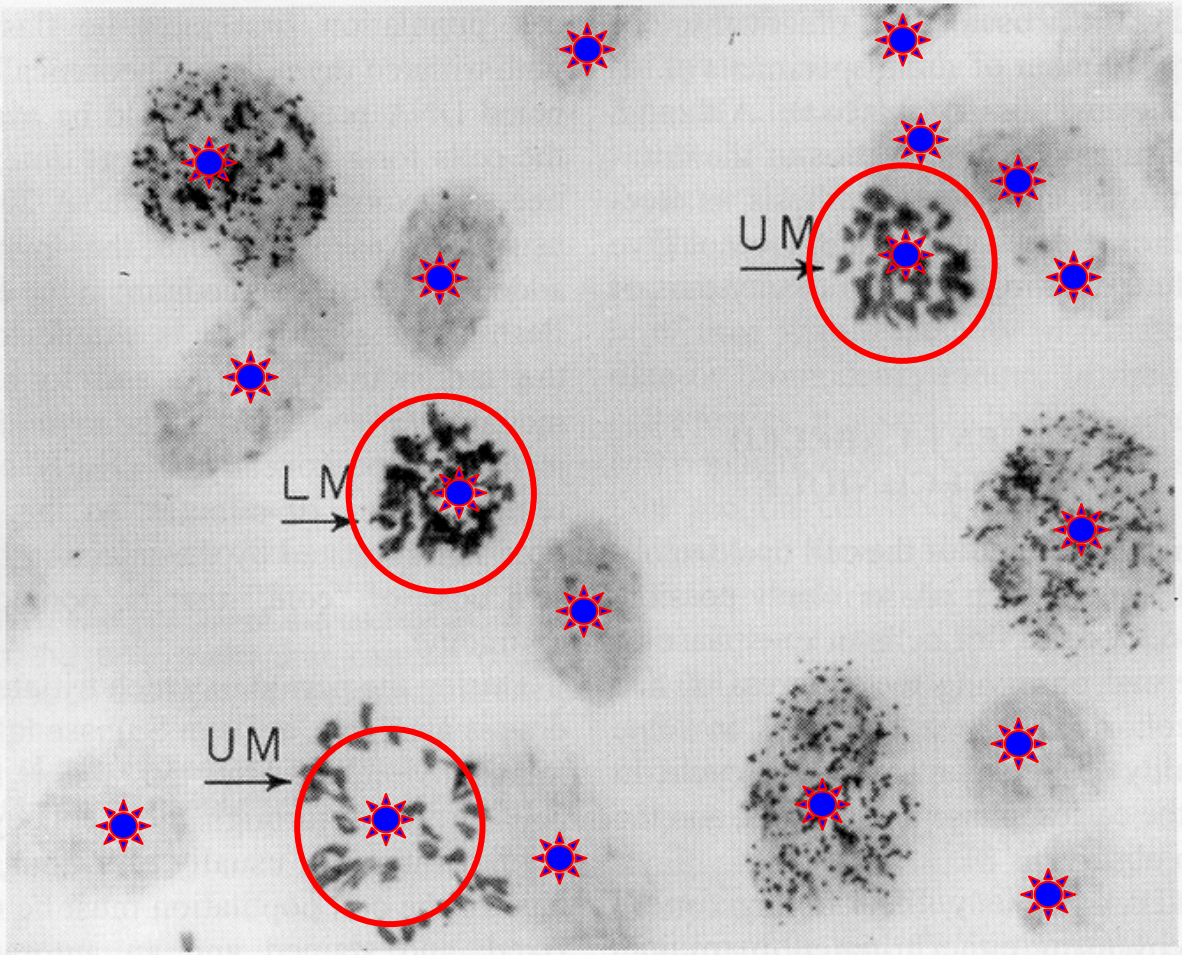


Figure 21.3. Photomicrograph of a preparation of mouse corneal cells. The cell preparation was flash-labeled some hours before with tritiated thymidine, which was taken up by cells in S. By the time the autoradiograph was made, the cell marked LM had moved around the cycle into mitosis; this is an example of a labeled mitotic figure. Other cells in mitosis are not labeled (UM). (Courtesy of Dr. M. Fry.)

M.I. = Tm/Tc

= λ Tm/Tc

M. I.

$$\frac{3}{18}$$

Mitotic Index (MI) cont.

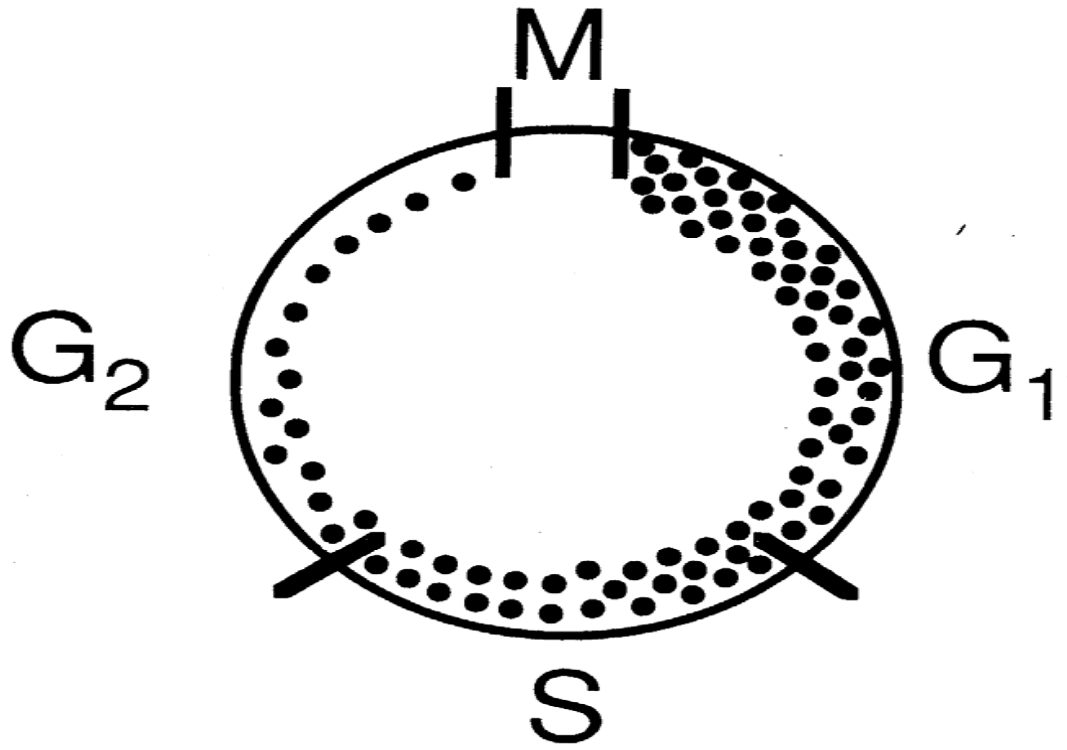


Figure 21.2. Diagram illustrating the fact that cells cannot be distributed uniformly in time around the cell cycle because they double in number during mitosis. The simplest assumption is that they are distributed as an exponential function of time.

Labeling Index (LI)

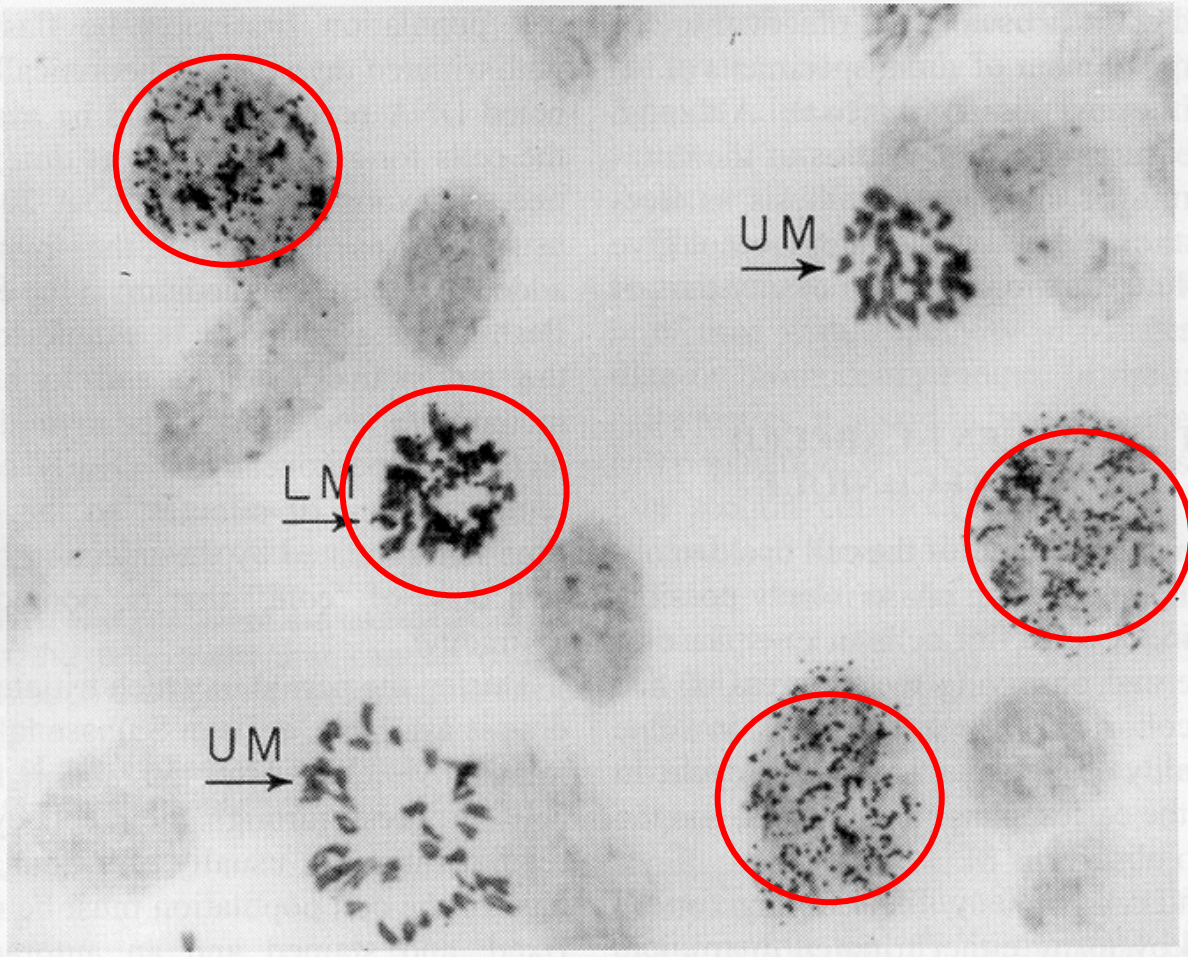


Figure 21.3. Photomicrograph of a preparation of mouse corneal cells. The cell preparation was flash-labeled some hours before with tritiated thymidine, which was taken up by cells in S. By the time the autoradiograph was made, the cell marked LM had moved around the cycle into mitosis; this is an example of a labeled mitotic figure. Other cells in mitosis are not labeled (UM). (Courtesy of Dr. M. Fry.)

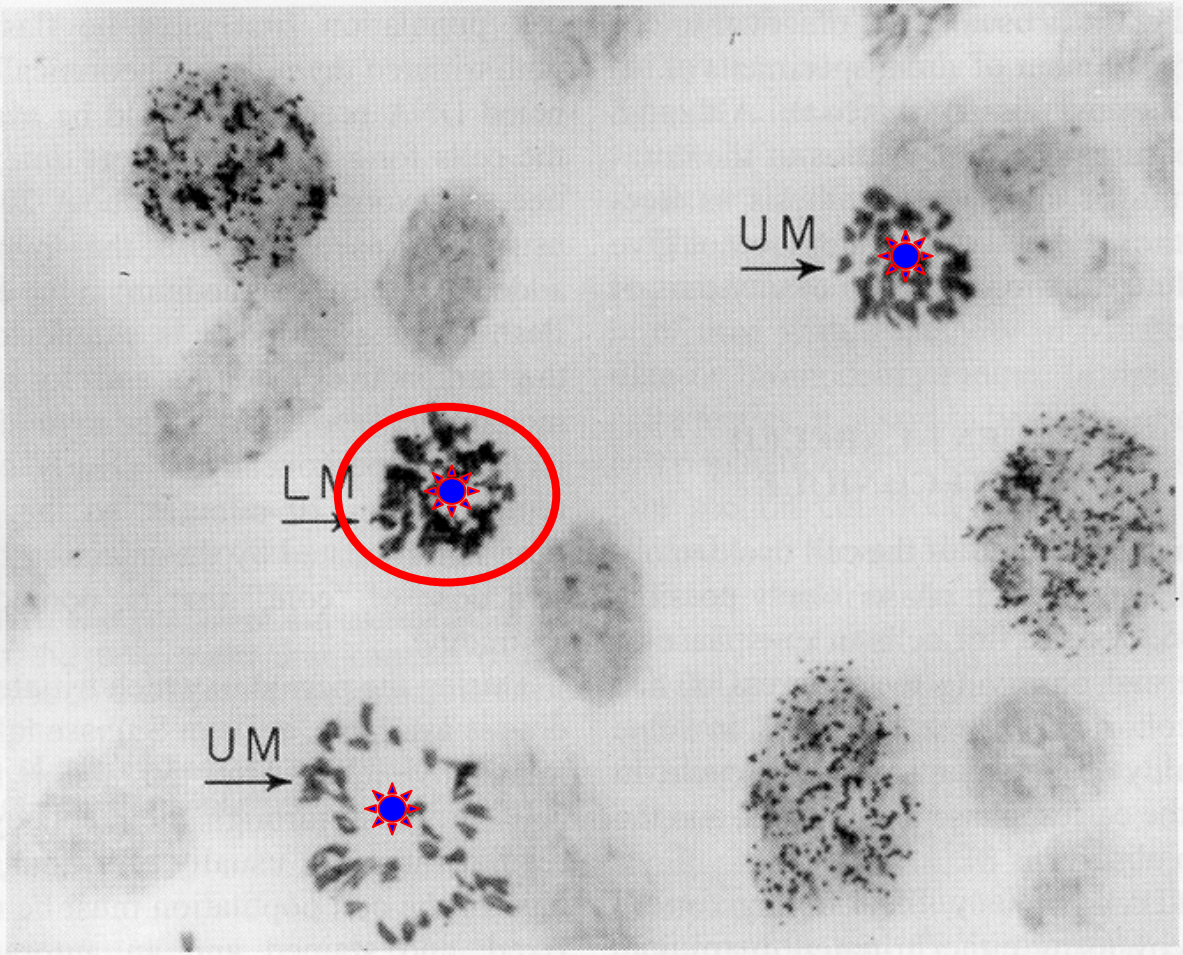
L.I.

$$\frac{4}{18}$$

$$L.I. = Ts/Tc$$

$$= \lambda\ Ts/Tc$$

Percent Labeled Mitosis (PLM)



PLM

$$\frac{1}{3}$$

Figure 21.3. Photomicrograph of a preparation of mouse corneal cells. The cell preparation was flash-labeled some hours before with tritiated thymidine, which was taken up by cells in S. By the time the autoradiograph was made, the cell marked LM had moved around the cycle into mitosis; this is an example of a labeled mitotic figure. Other cells in mitosis are not labeled (UM). (Courtesy of Dr. M. Fry.)

Percent Labeled Mitoses

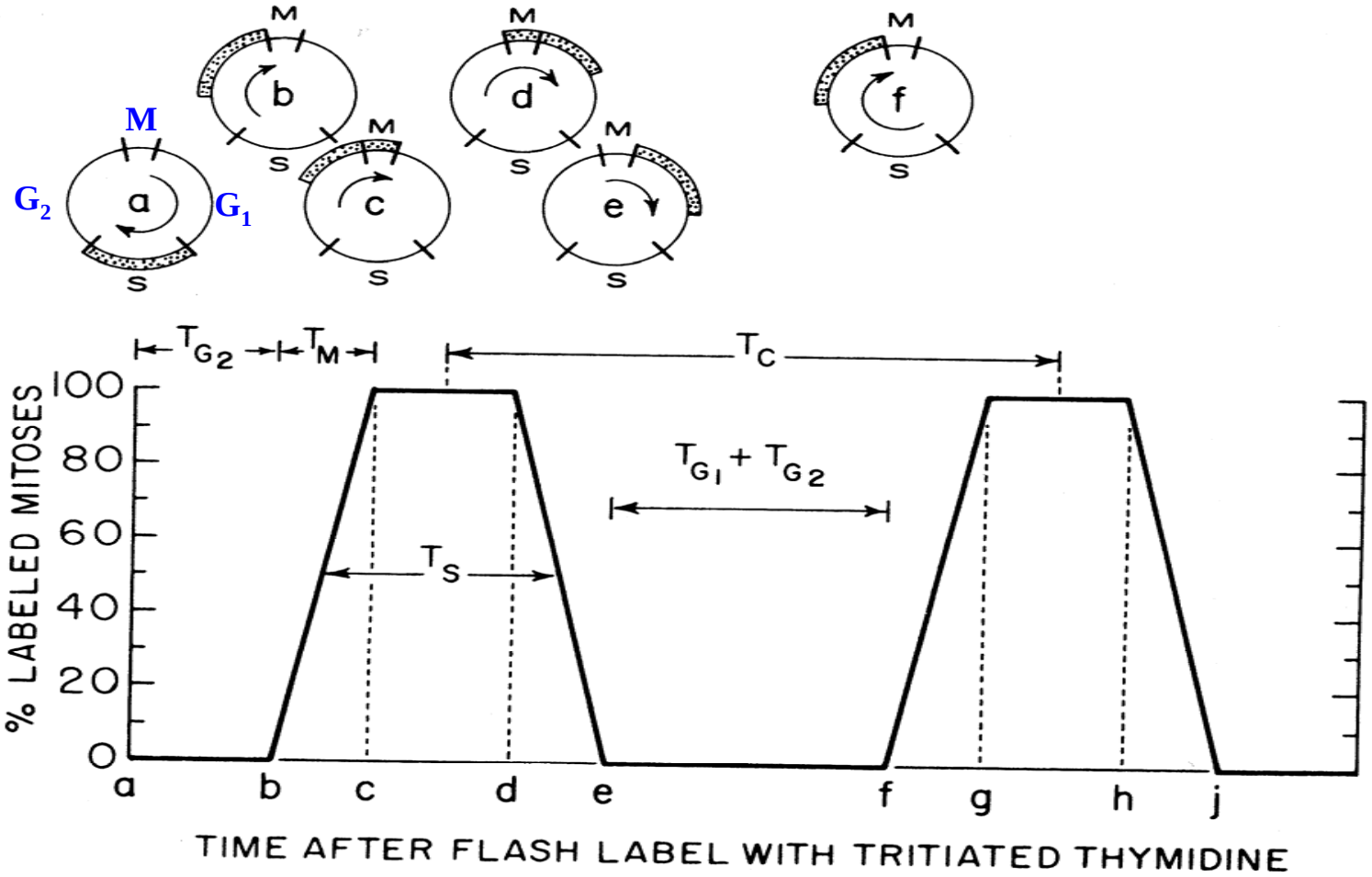


Figure 21.4. Percent-labeled mitoses curve for an idealized cell population in which all of the cells have identical mitotic cycle times. The cell population is flash-labeled with tritiated thymidine, which labels all cells in S. The proportion of labeled mitotic cells is counted as a function of time after labeling. The circles at the top of the figure indicate the position of the labeled cohort of cells as it progresses through the cycle. The length of the various phases (*e.g.*, T_{G2}, T_M) of the cycle (T_C) may be determined as indicated.

Percent Labeled Mitoses (cont.)

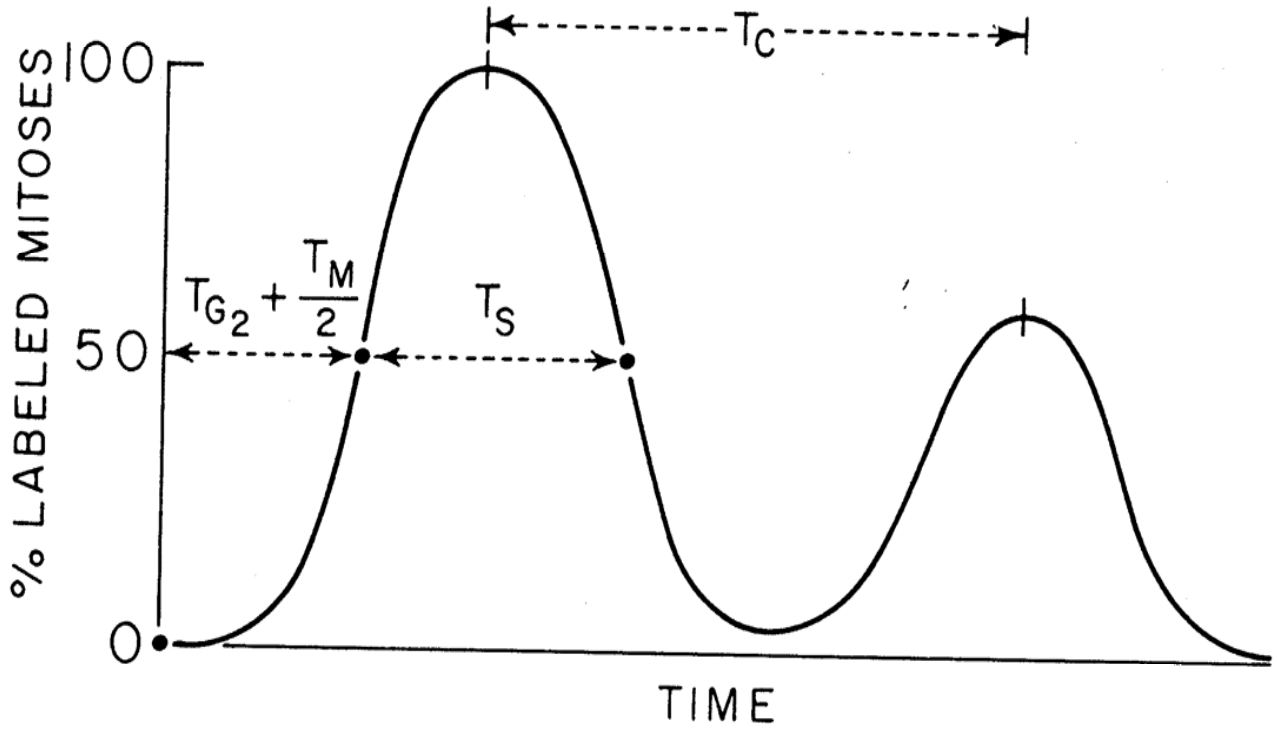


Figure 21.5. Typical percent-labeled mitoses curve obtained in practice for the cells of a tissue or tumor. It differs from the idealized curve in Figure 21.4 in that the only points that can be identified with precision are the peaks of the curve and the 50% levels. The first peak is symmetric, and the second peak is lower than the first because the cells of a population have a range of cell cycle times.

Percent Labeled Mitoses (cont.)

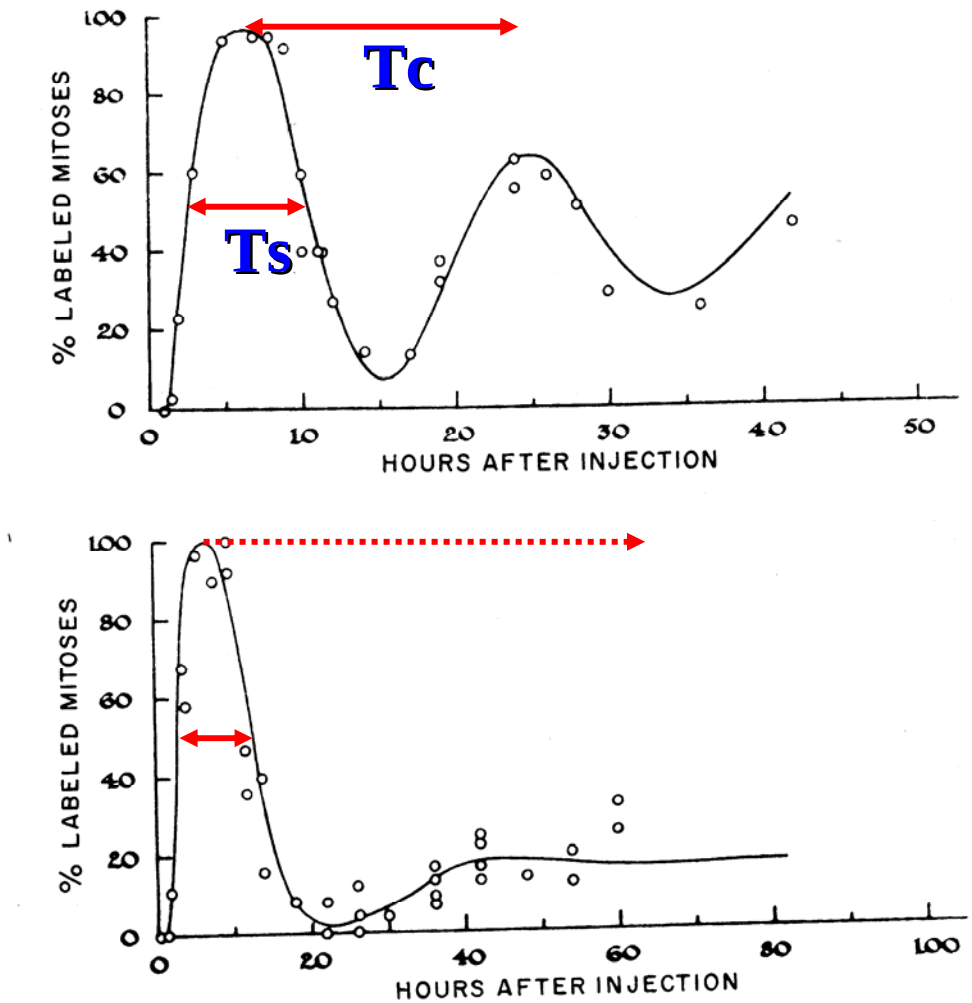


Figure 21.6. Percent-labeled mitoses curve for two transplantable rat sarcomas with widely different growth rates. The tumor in the **upper panel** has a gross doubling time of 22 hours, compared with 190 hours for the tumor in the **lower panel**. (From Steel GG, Adams K, Barratt JC: Analysis of the cell population kinetics of transplanted tumours of widely differing growth rate. Br J *Cancer* 20:784–800, 1966, with permission.)

Percent Labeled Mitoses (cont.)

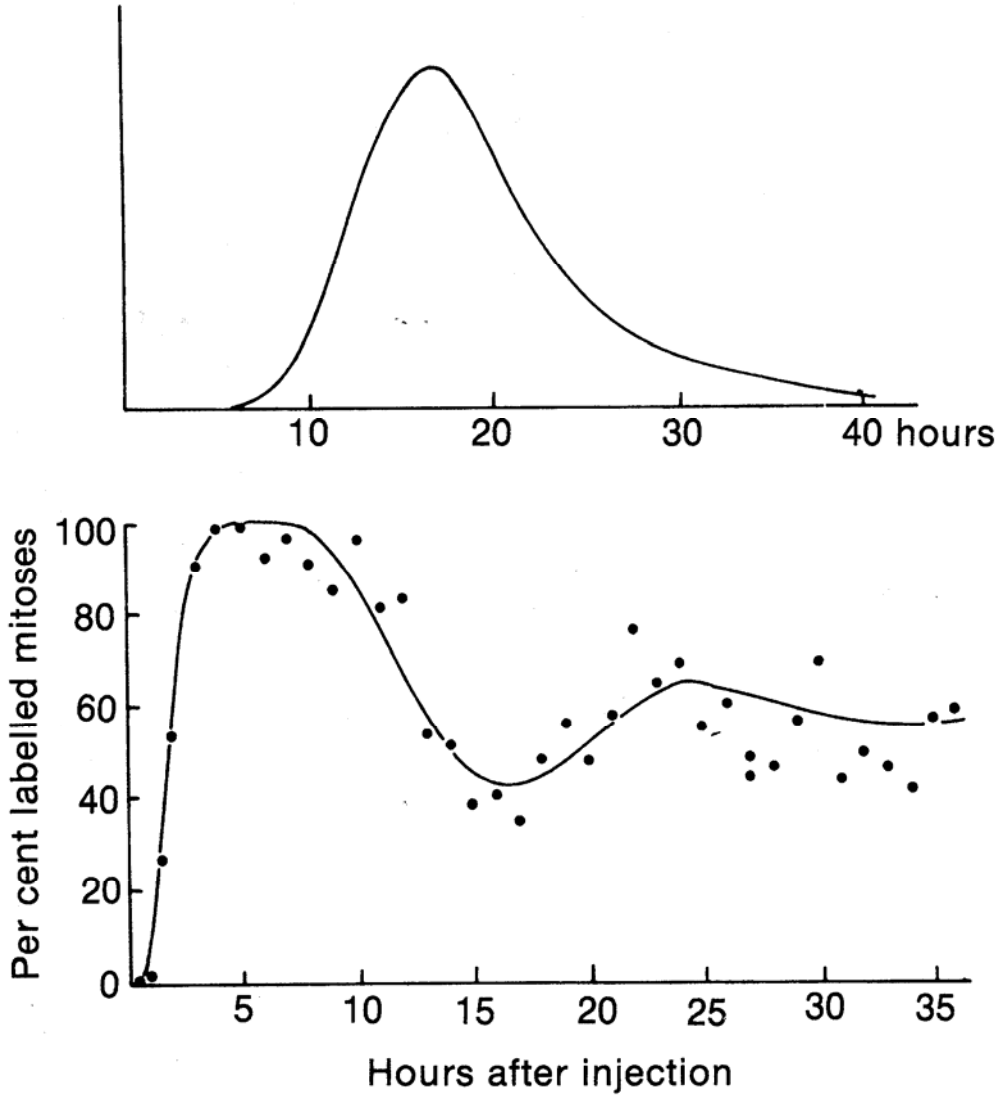


Figure 21.7. Bottom: Percent-labeled mitoses curve for an EMT6 mouse tumor. (Data from Dr. Sara Rockwell.) **Top:** The distribution of cell-cycle times consistent with the damped labeled mitoses curve, obtained by computer analysis of the data and a mathematic model. (From Steel GG: Laryngoscope 85:359–370, 1975, with permission.)

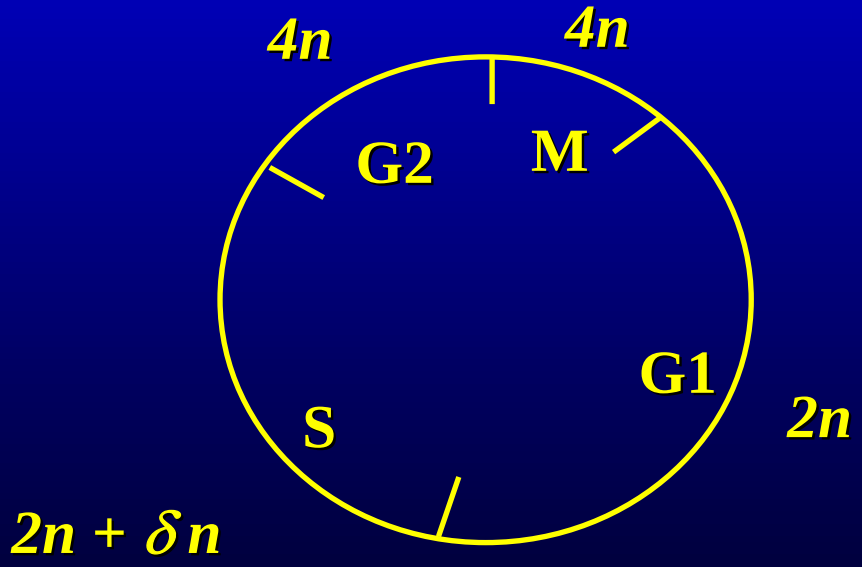
Table 21.1

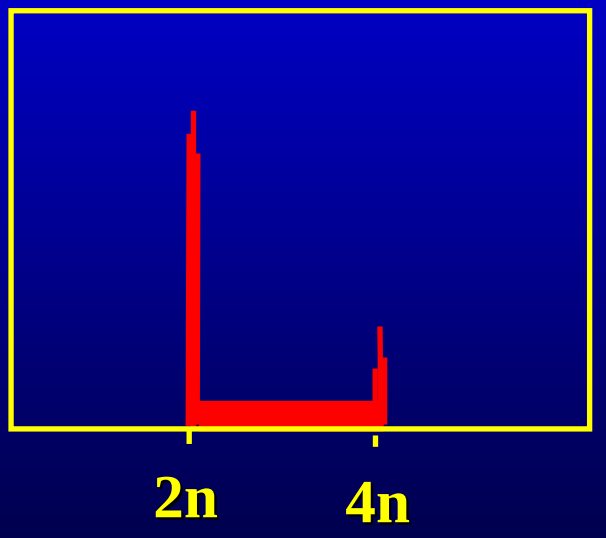
TABLE 21.1. *The Constituent Parts of the Cell Cycle for Some Cells in Culture and Tumors in Experimental Animals*

Authors	Cell or Tissue	T_C, h	T_S, h	T_M, h	T_{G2}	T_{G1}
Bedford	Hamster cells *in vitro*	10	6	1	1	2
	HeLa cells *in vitro*	23	8	1	3	11
Steel	Mammary tumors in the rat					
	BICR/M1	19	8	~1	2	8
	BICR/A2	63	10	~1	2	50
Quastler and Sherman	Mouse intestinal crypt	18.75	7.5	0.5	0.5–1.0	9.5
Brown and Berry	Hamster cheek pouch epithelium	120–152	8.6	1.0	1.9	108–140
	Chemically induced carcinoma in pouch	10.7	5.9	0.4	1.6	2.8

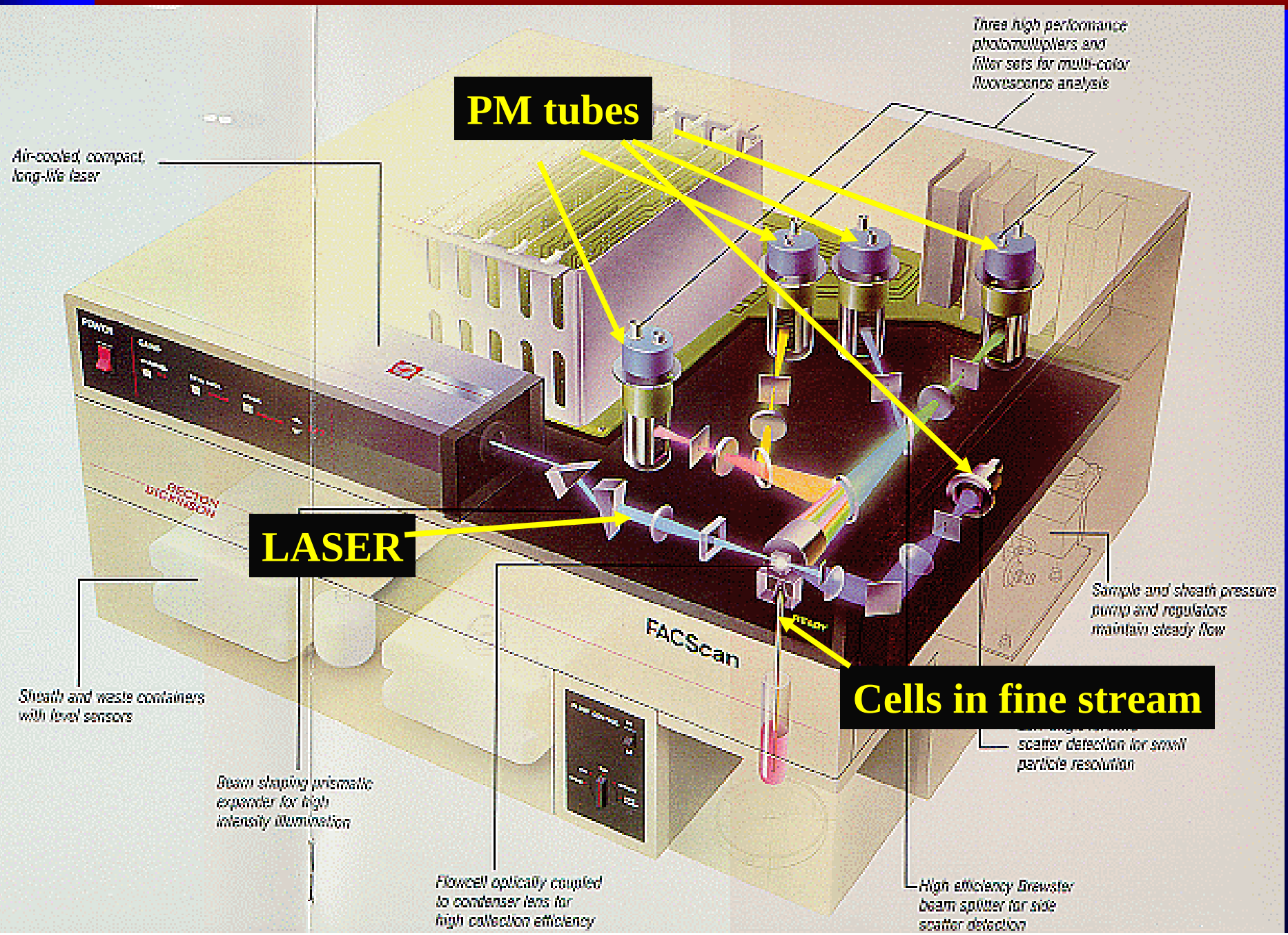
Cell Cycle Analysis by Flow Cytometry

1. label DNA with propidium iodide (fluorescent dye)
2. measure light output by flow cytometry
3. analyze DNA histograms



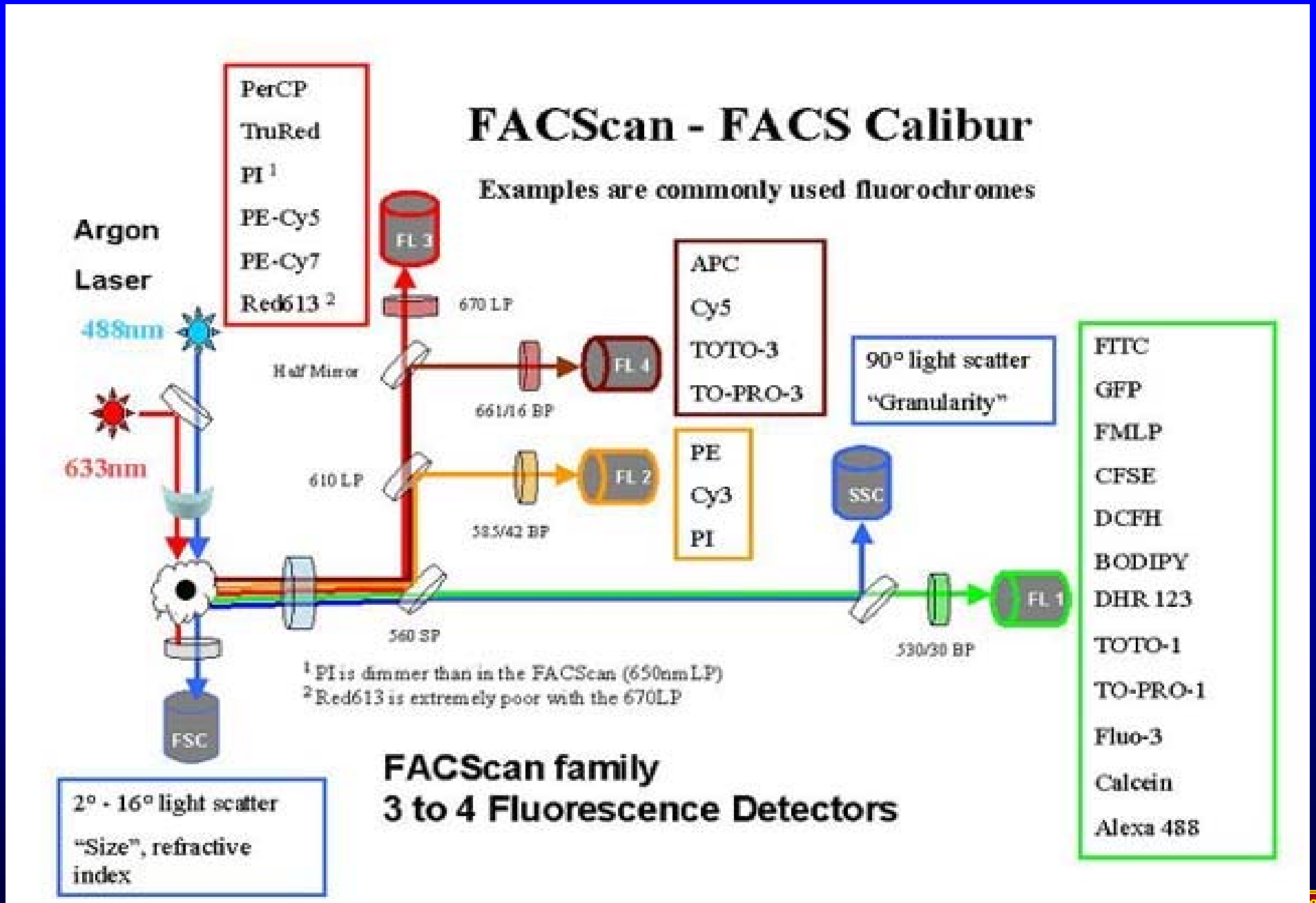


FACS



Most cell cycle work now uses flow cytometry where a laser excites dye in cells and output is collected by photomultiplier tubes.DNA can be labeled by propidium iodide (P.I.) and S phase cells by BrdUrd (detected using a fluorescent antibody)

Fluorescence Activated Cell Sorting



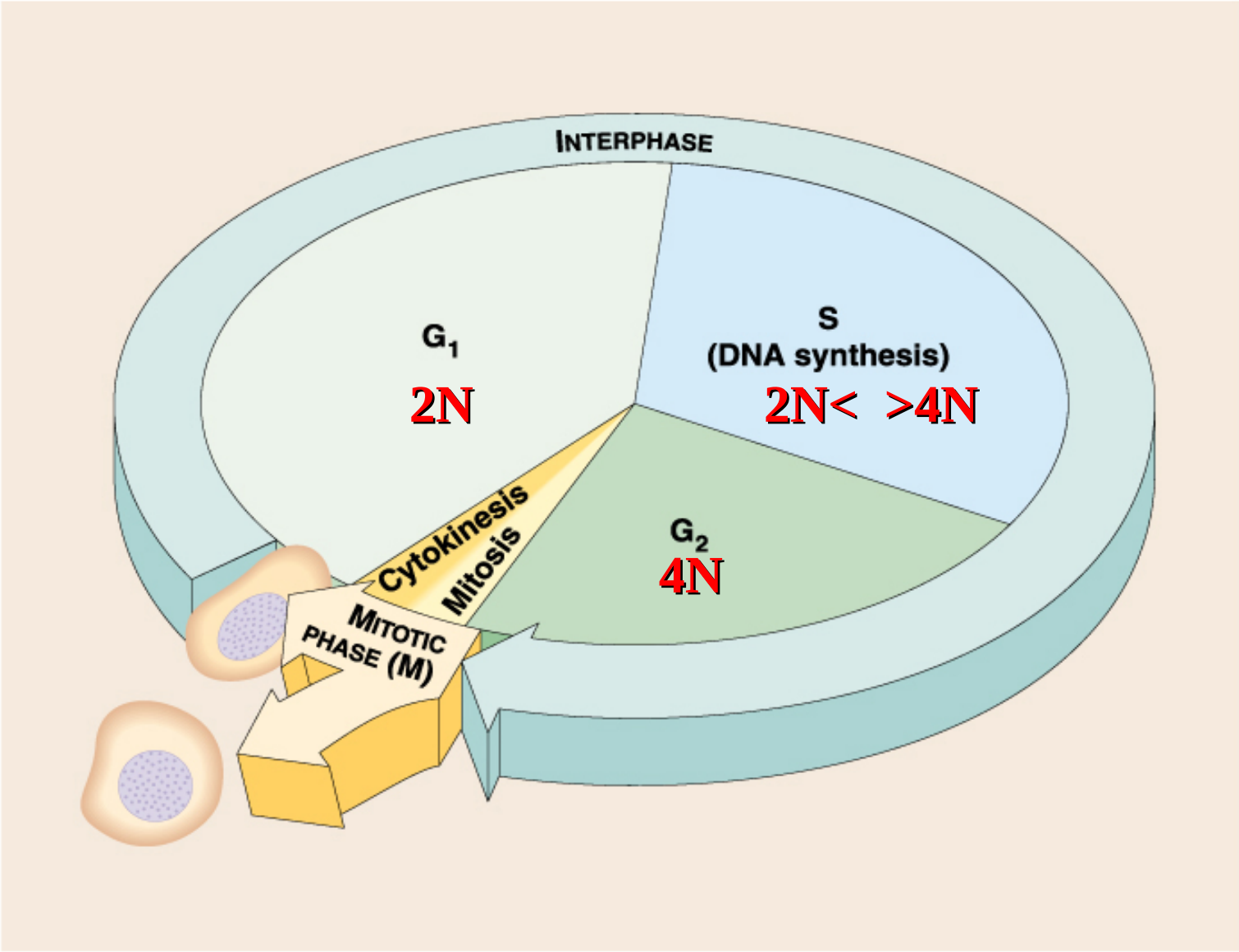
Principles of Using FACS

- Size difference (FSC)
- Granularity difference (SSC)
- Binding ability to fluorochromes
  - FL1
  - FL2
  - FL3
  - FL4

Applications of FACS

- Cell cycle analysis
- Chromosome analysis (DNA analysis)
- Cell Sorting
- Cell Phenotyping
- Apoptosis
- Functional studies

Cell Cycle



Cell Cycle (DNA) Analysis

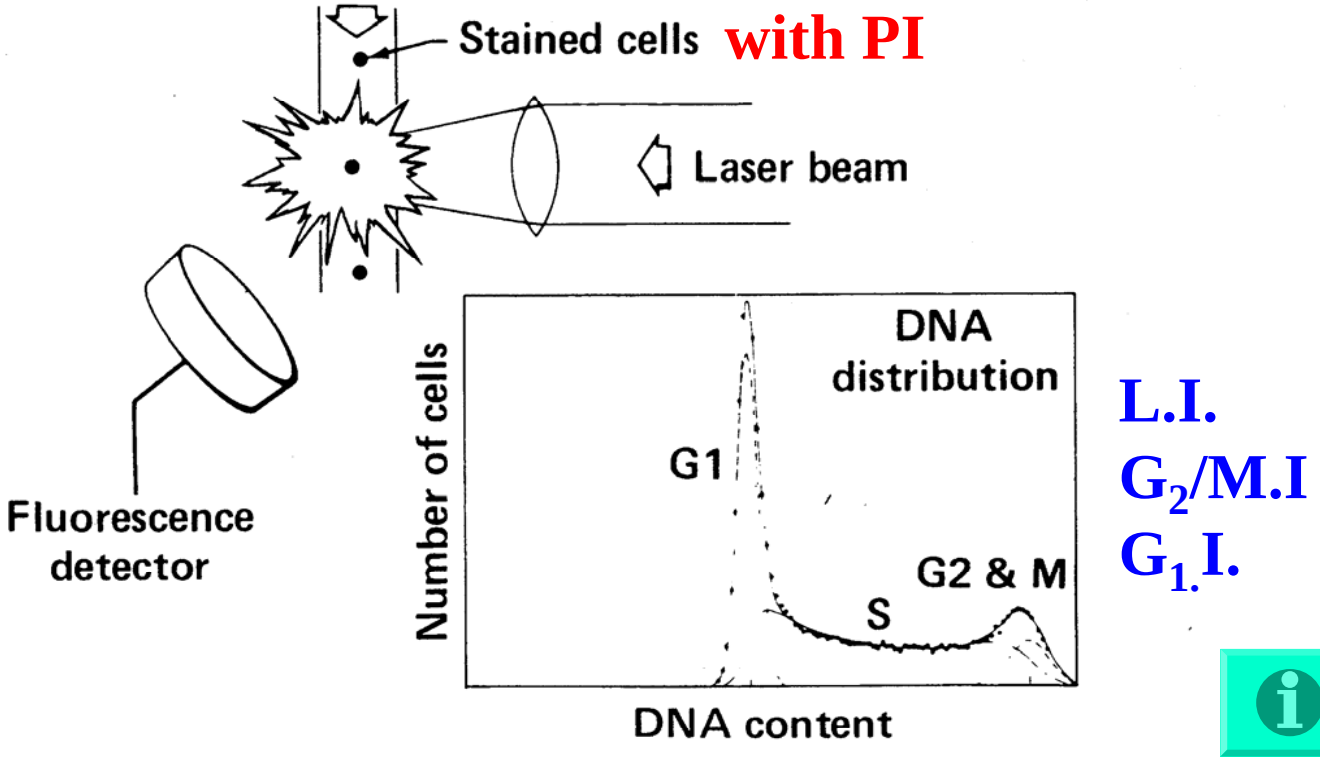
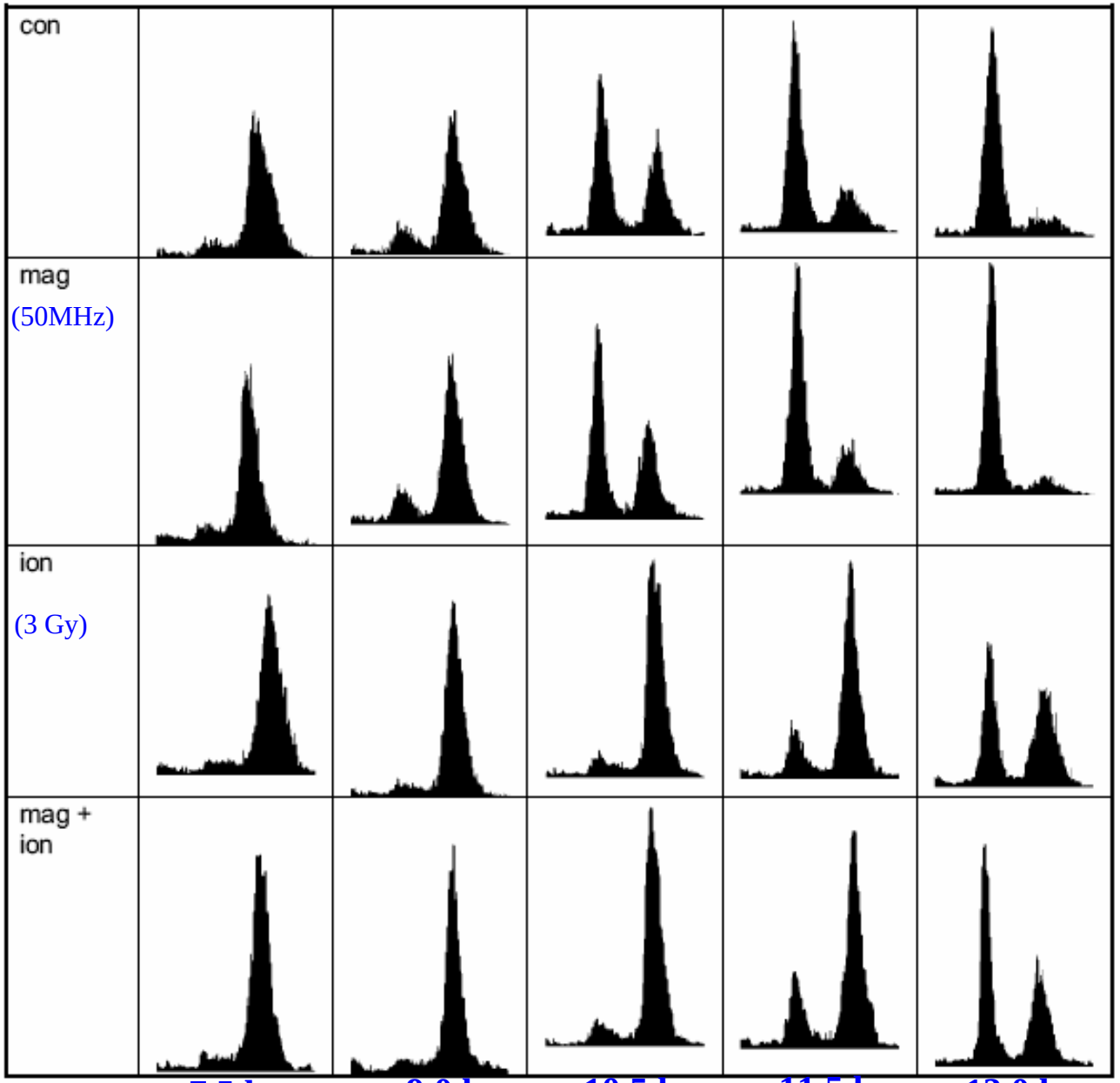
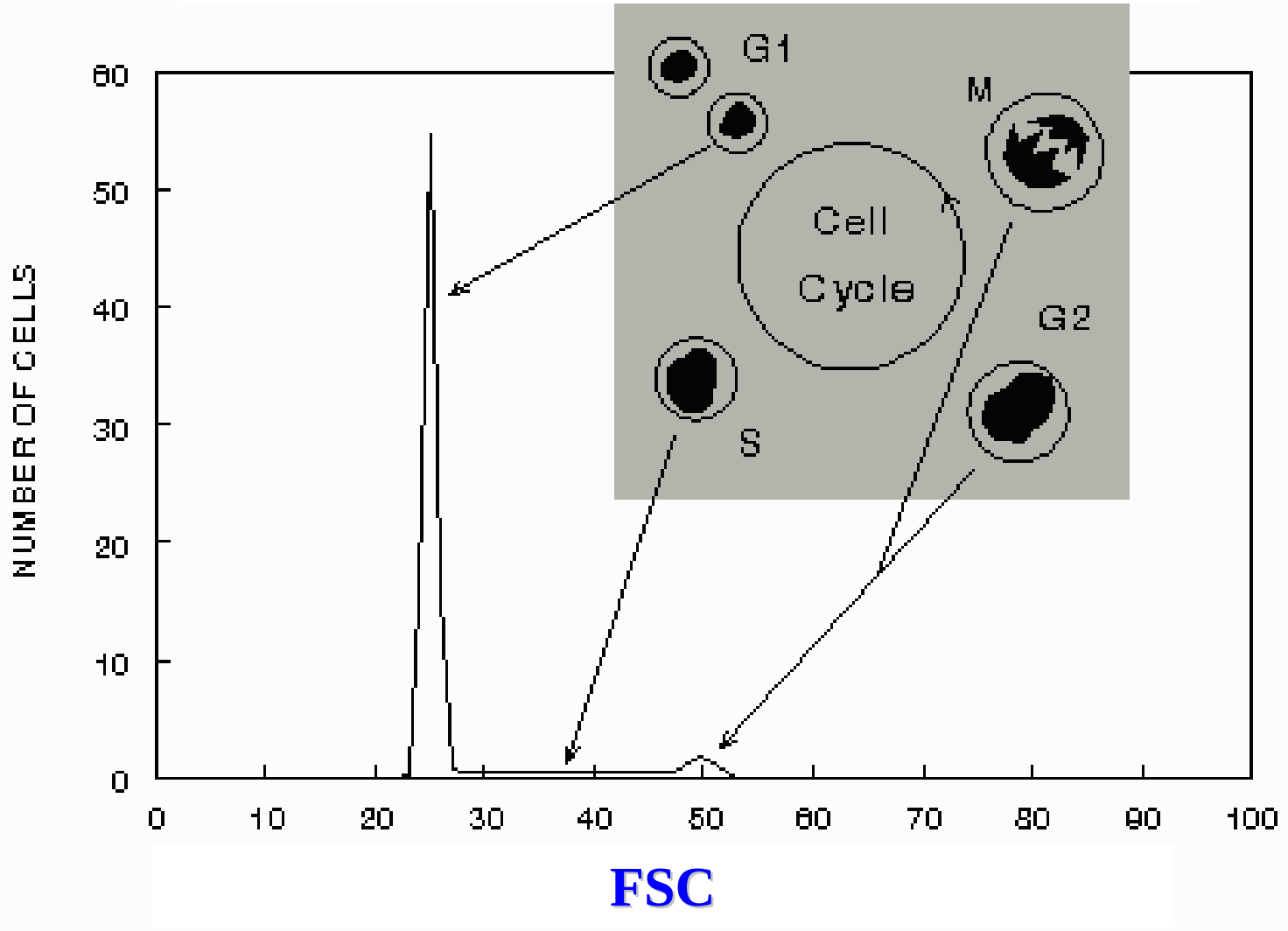


Figure 21.8. The principles of DNA distribution analysis of flow cytometry. Suspensions of fluorescent-stained single cells flow one at a time through a light beam with its wavelength adjusted to excite the fluorescent dye. The fluorescence stimulated in each cell is recorded as a measure of that cell's DNA content. Thousands of cells can be measured each second and the results accumulated to form a DNA distribution like that shown for asynchronously growing Chinese hamster ovary cells. (From Gray JW, Dolbeare F, Pallavicini MG, Beisker W, Waldman F: Cell cycle analysis using flow cytometry. Int J Radiat Biol 49:237–255, 1986, with permission.)

RT-induced G_2/M block



Cell Cycle: Cell Size Analysis



Cell Cycle: DNA Analysis II

Cell
+
BrdUTP

+
Ab-BrdUTP
|
FITC

FACS

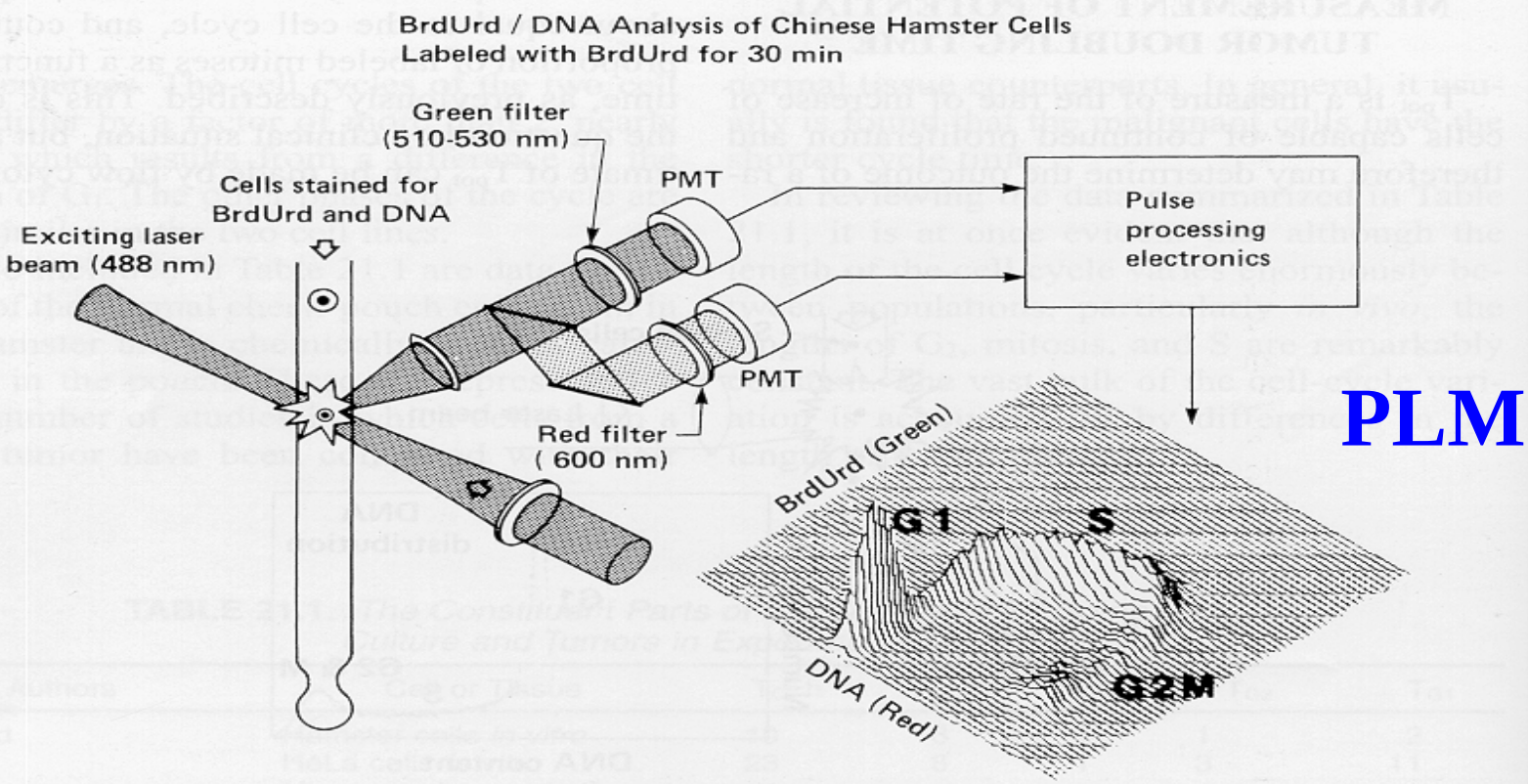
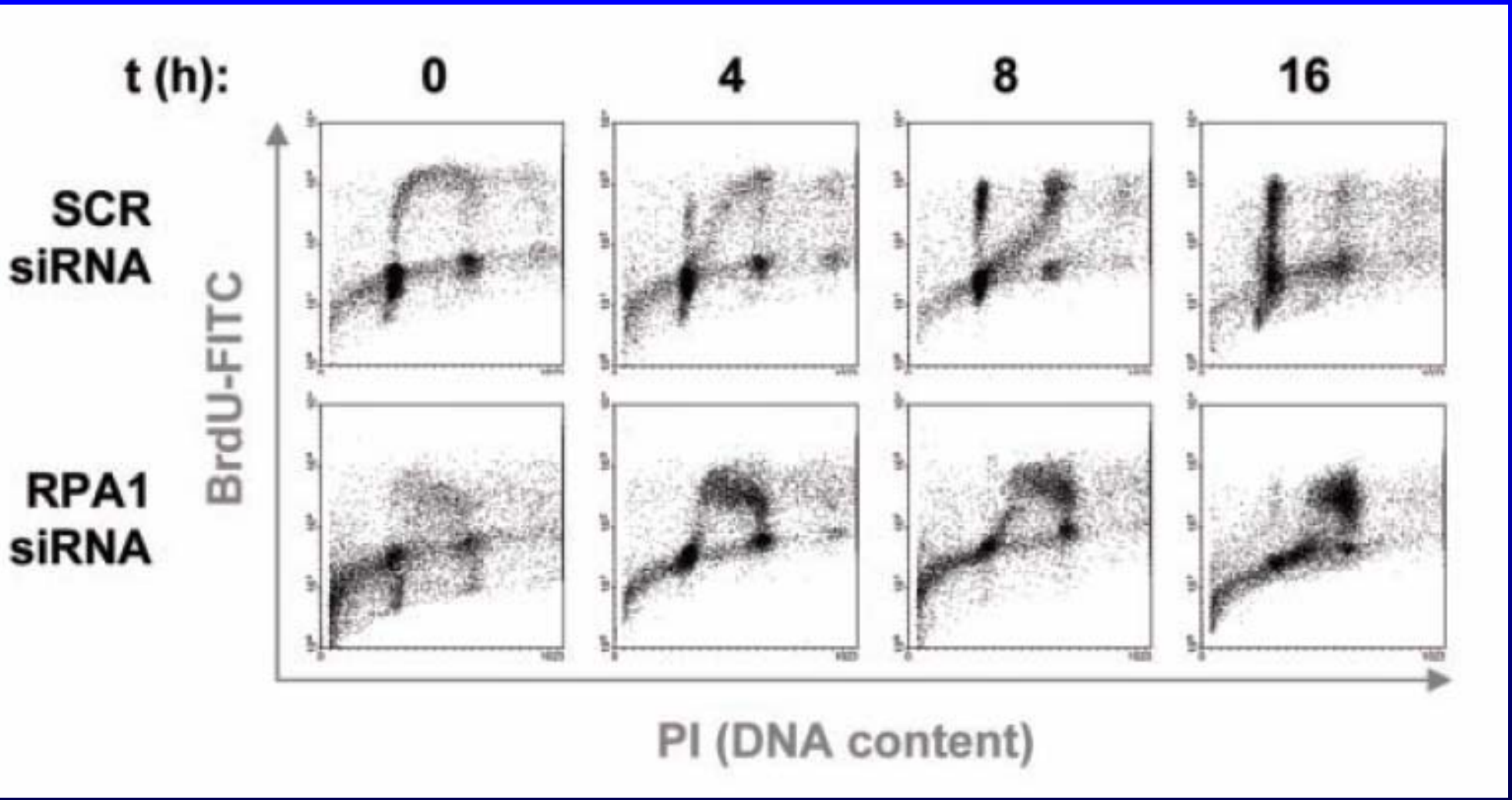
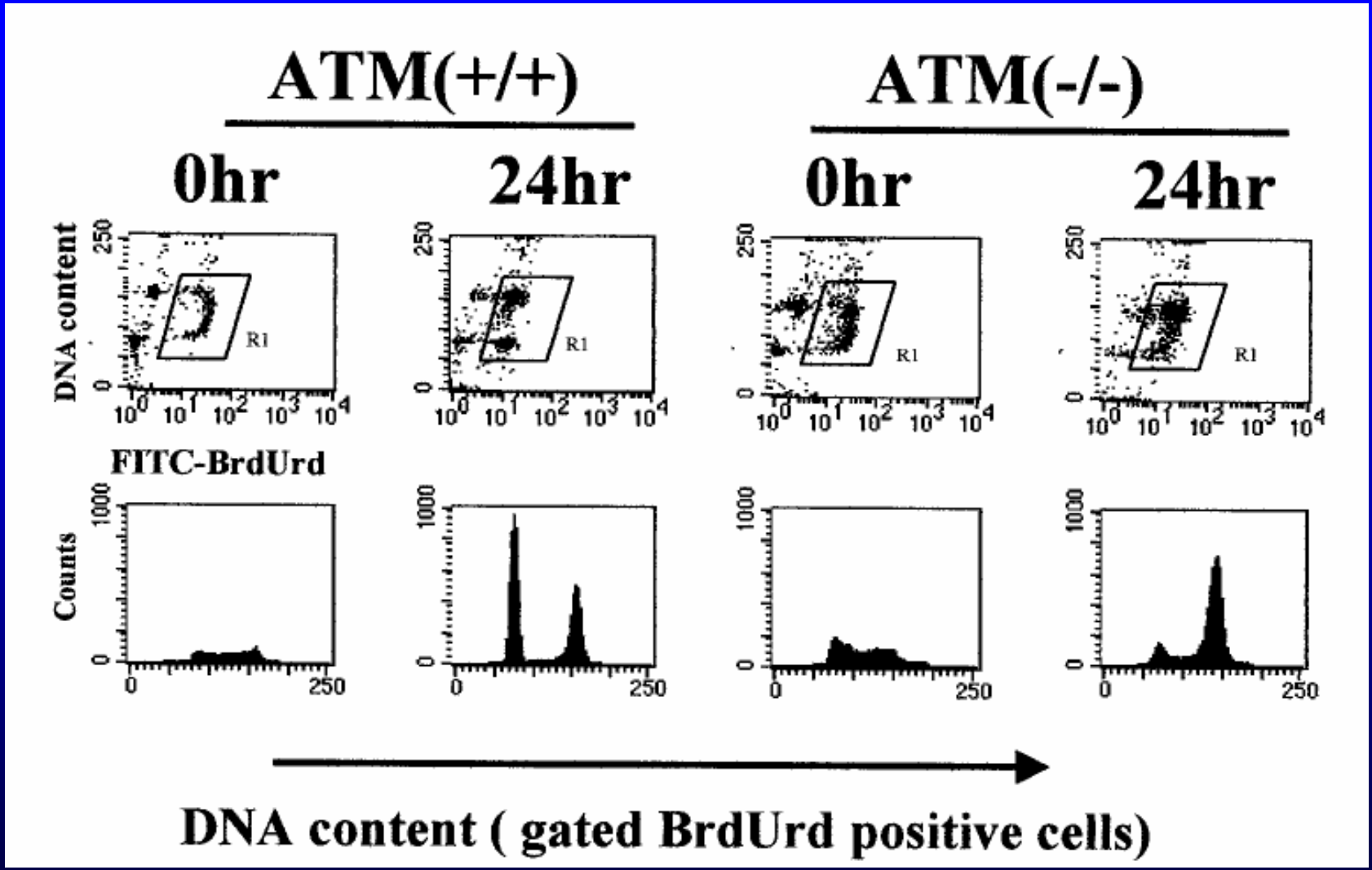


Figure 21.9. The flow cytometric analysis of cellular bromodeoxyuridine (BrdUrd) and DNA content for cells stained with fluorescein (linked to BrdUrd) and propidium iodide (linked to DNA). The cells are processed one at a time through a blue (488-nm) laser beam that excites cellular BrdUrd content, and red fluorescence is recorded as a measure of cellular DNA content. The BrdUrd (green fluorescence) axis in the bivariate is logarithmic, with every seven channels representing a doubling of fluorescence intensity. (From Gray JW, Dolbeare F, Pallavicini MG, Beisker W, Waldman F: Cell cycle analysis using flow cytometry. Int J Radiat Biol 49:237–255, 1986, with permission.)

Cell cycle detection



RT-induced cell cycle delay



FACS - Ts

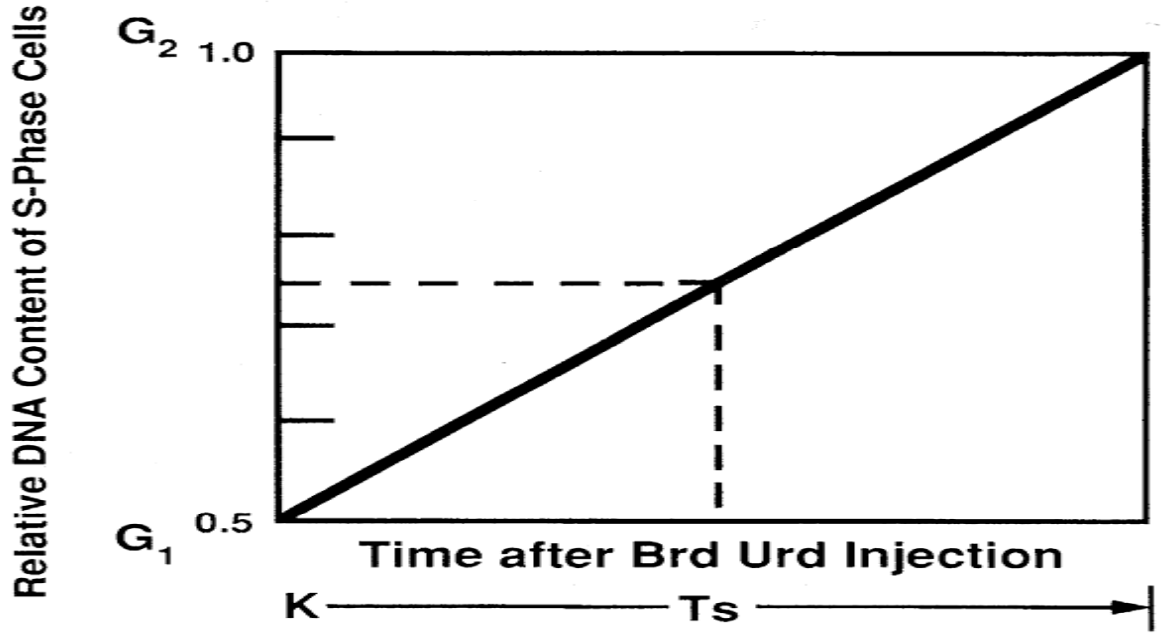
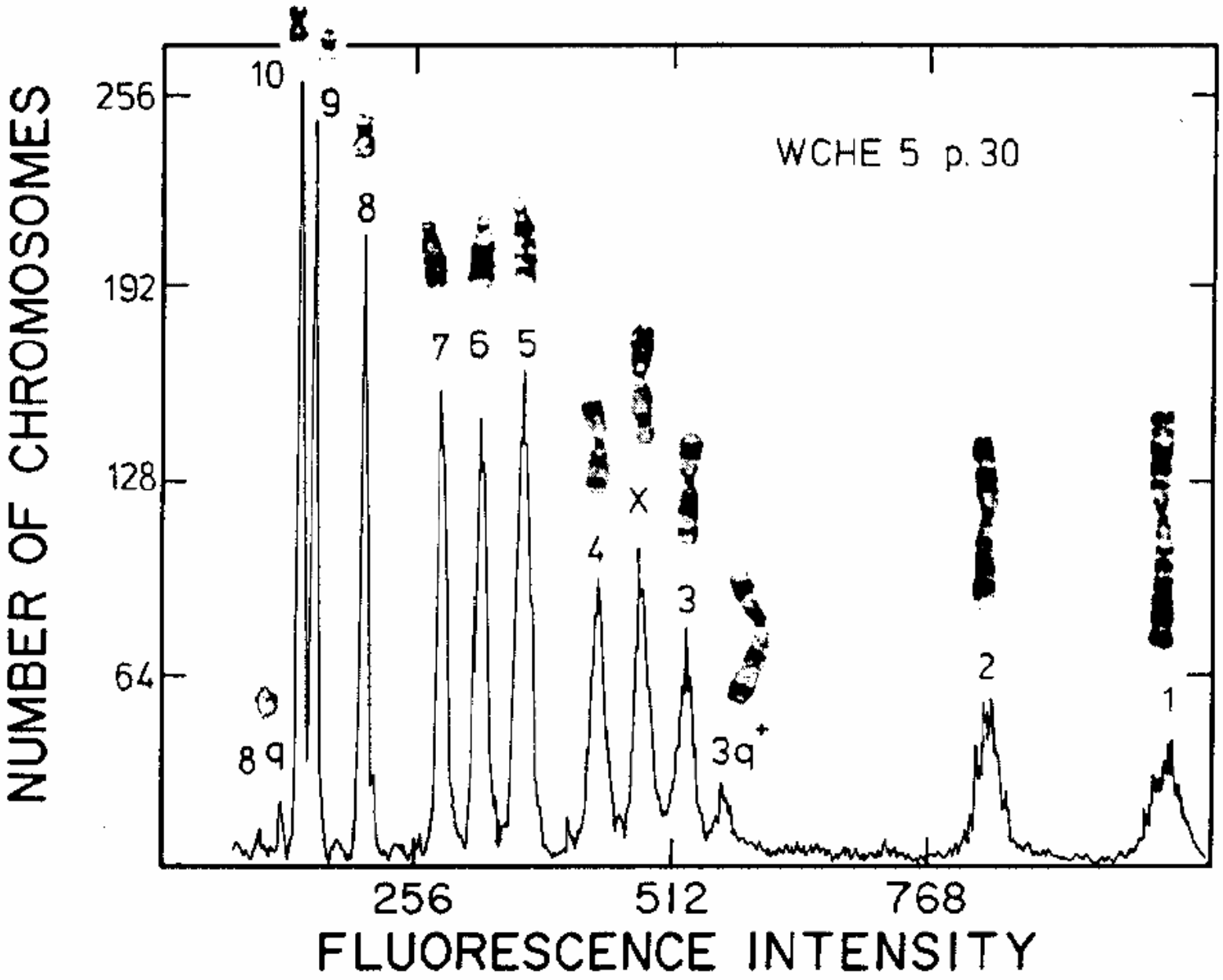


Figure 21.10. Graph illustrating the way in which T_S can be estimated by flow cytometry on cells from a single tumor biopsy specimen taken 4 to 8 hours after an injection of a thymidine analogue (bromodeoxyuridine or iododeoxyuridine). Cells in S phase are identified by the green fluorescence from an antibody to the thymidine analogue. The relative DNA content is measured by the red fluorescence owing to the incorporated propidium iodide. The DNA content in G_2 cells is double that in G_1. The length of the DNA synthetic phase (T_S) can be estimated by the relative DNA content of the S-phase cells in relation to the time between the injection of the thymidine analogue and the biopsy.

Functional Studies of FACS

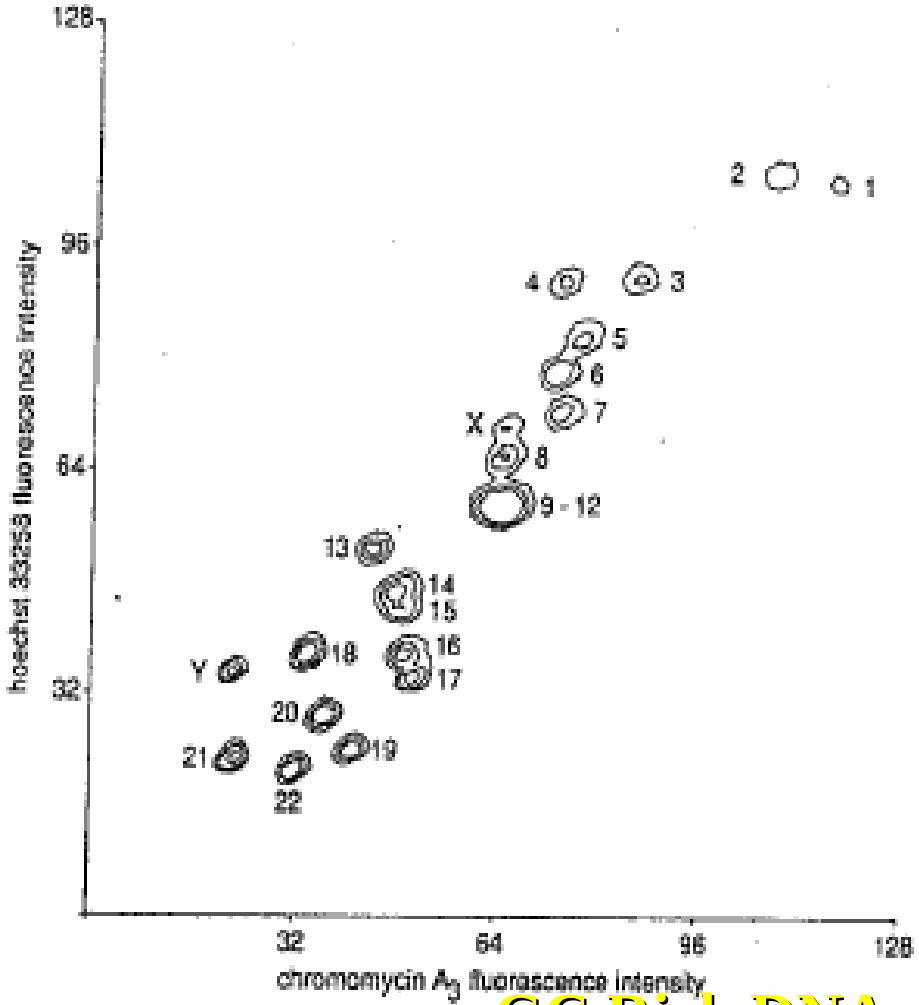
- Measurement of Calcium Flux
- pH Measurement
- Measurement of Reactive Oxygen
- Measurement of Intracellular Glutathione
- Measurement of Membrane potential
- Phagocytosis
- Measurement of Green Fluorescent Protein
- Membrane polarization

Chromosome Analysis I

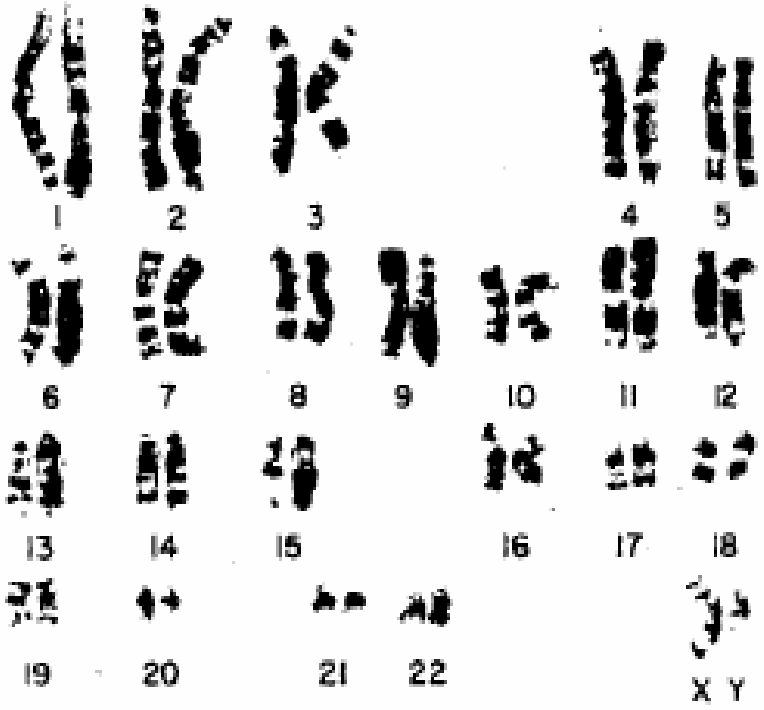


Chromosome Analysis II

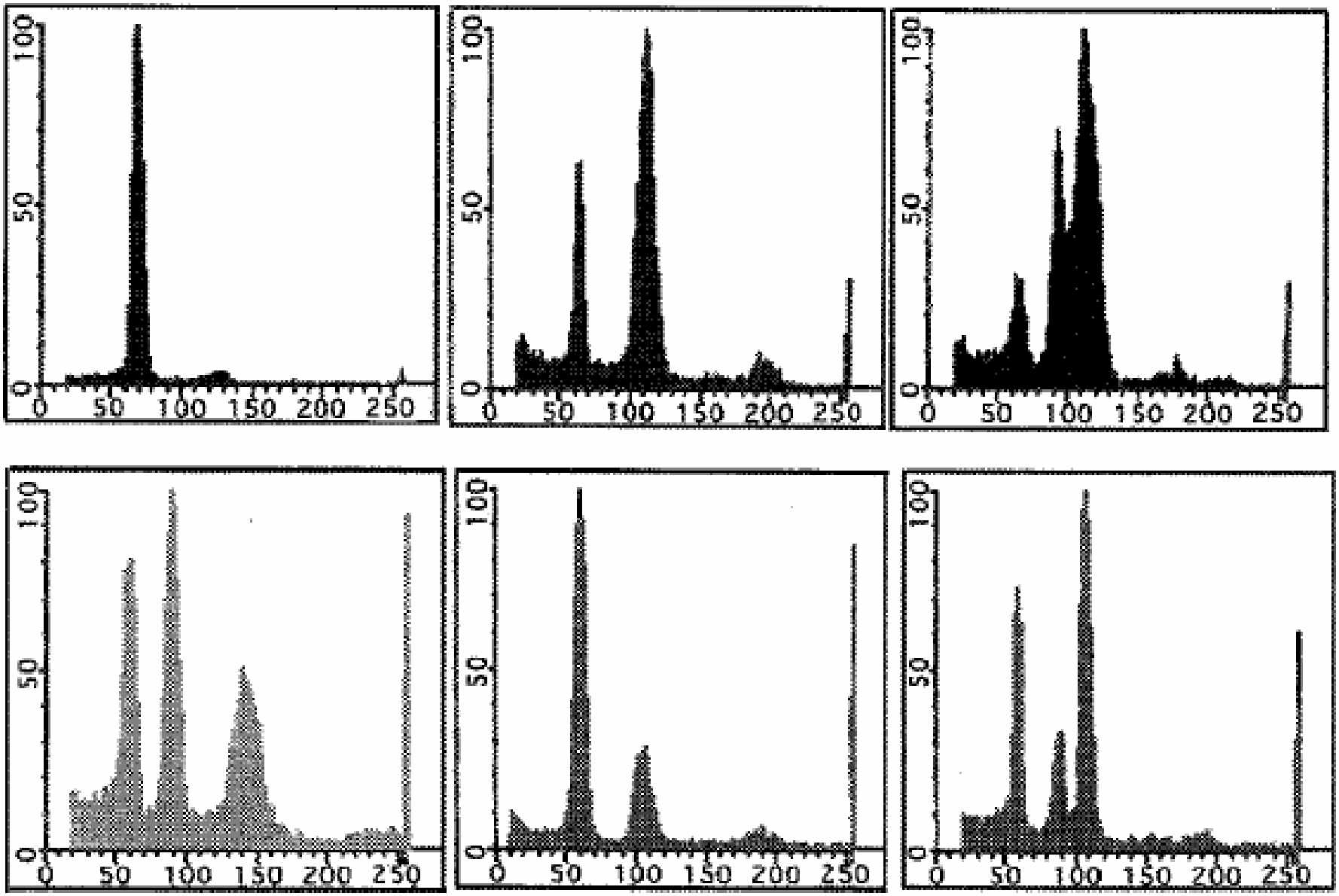
AT Rich DNA



GC Rich DNA

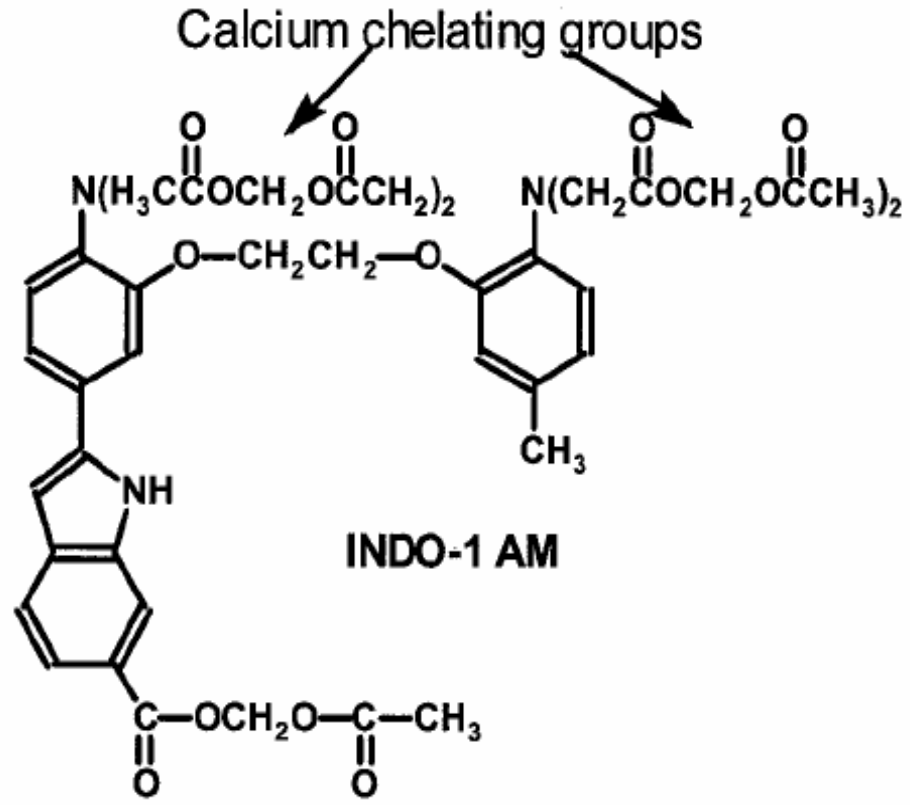


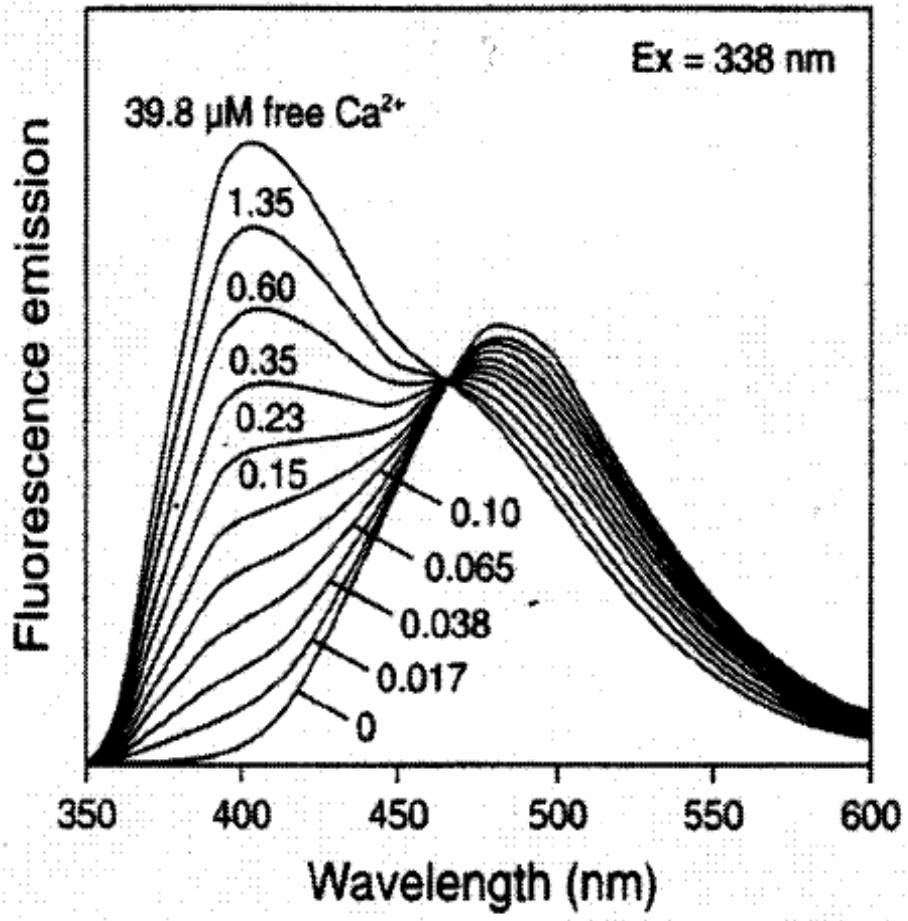
DNA Ploidy



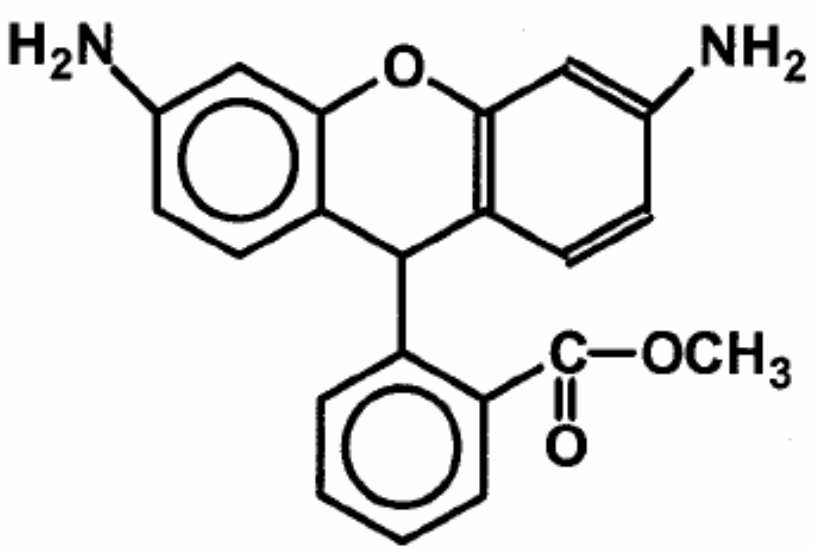
Measurement of [Ca^{++}]

Structure and emission spectra of indo-1





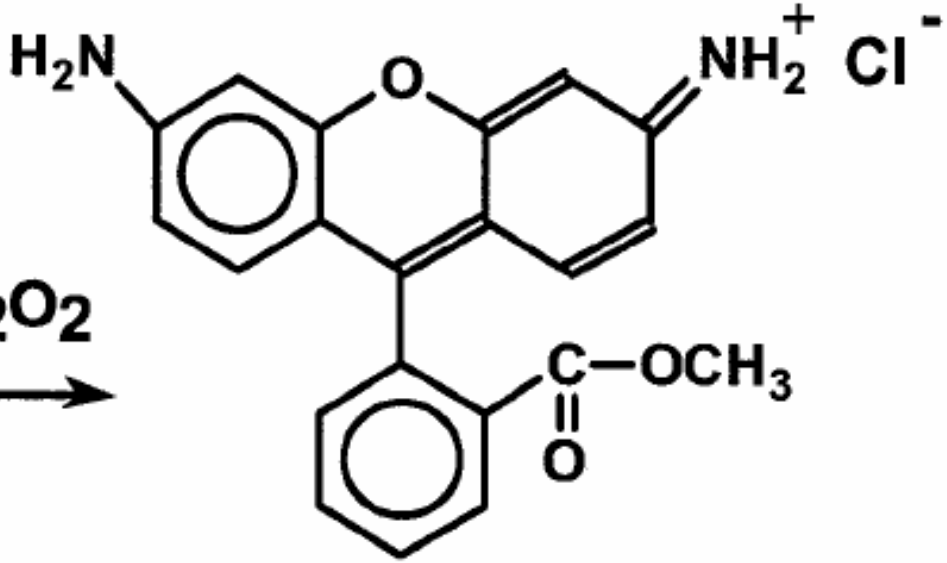
Reactive Oxygen



DIHYDRORHODAMINE 123

(Non-fluorescent)

H_2O_2

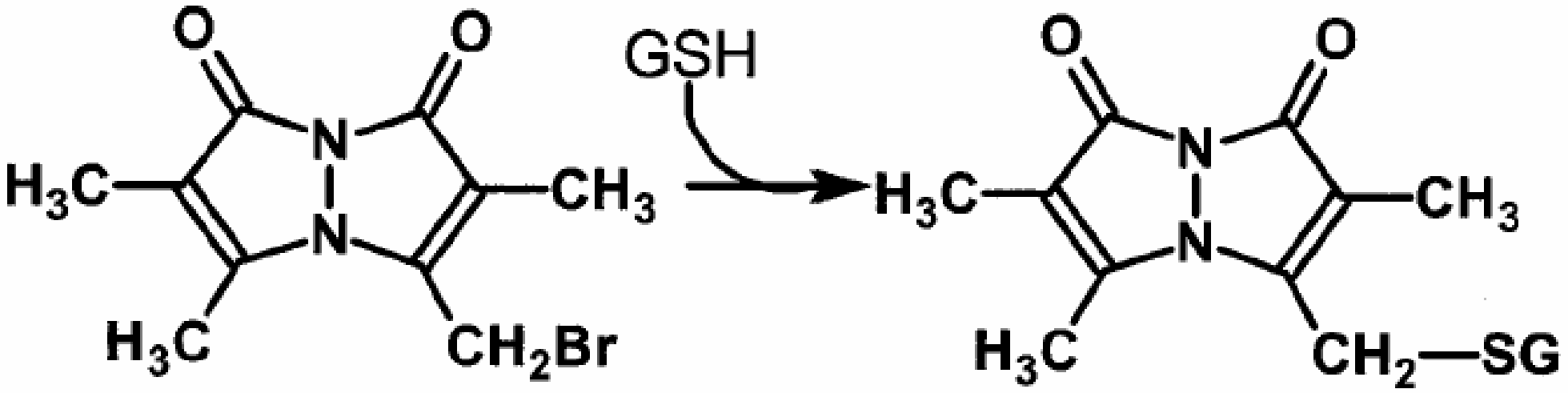


RHODAMINE 123

(Fluorescent)

Glutathione

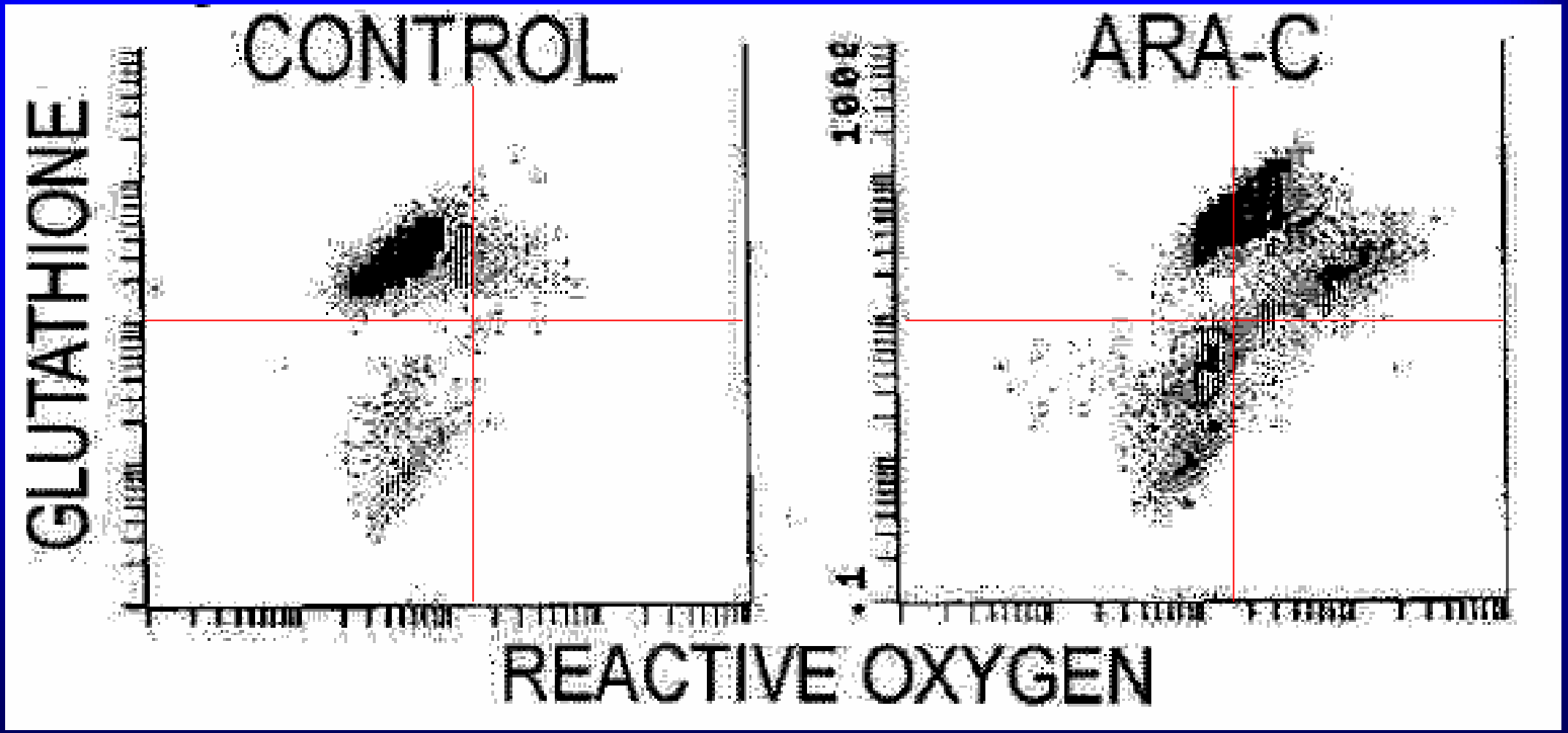
REACTION OF MONOBROMOBIMANE WITH GSH



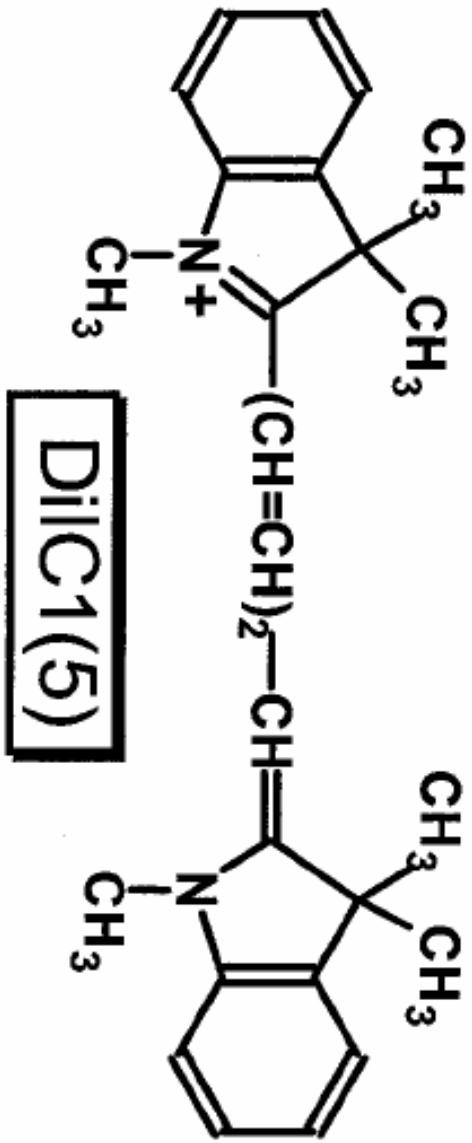
NON-FLUORESCENT

HIGHLY FLUORESCENT

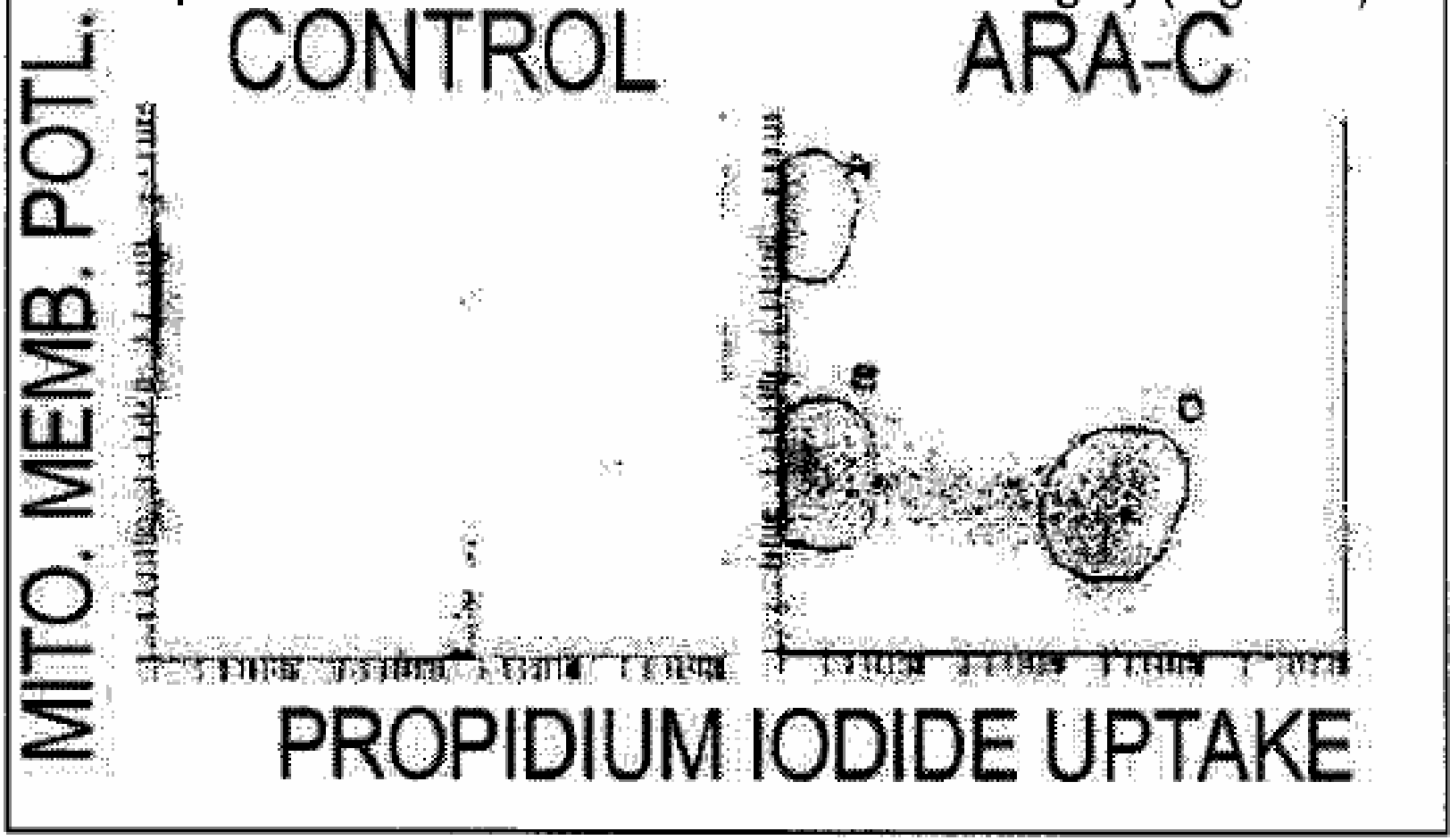
FACS for [H_2O_2] & [Glutathione]



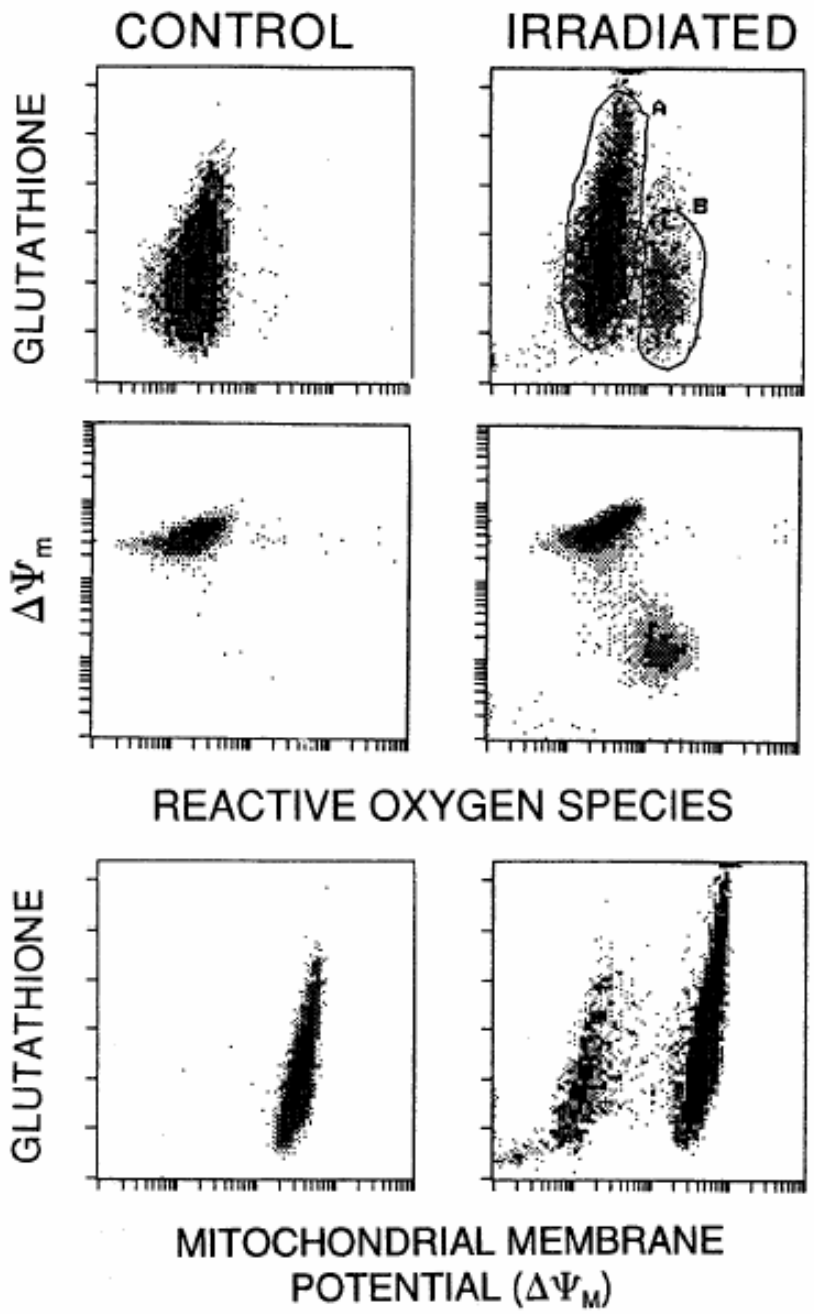
Membrane Potential



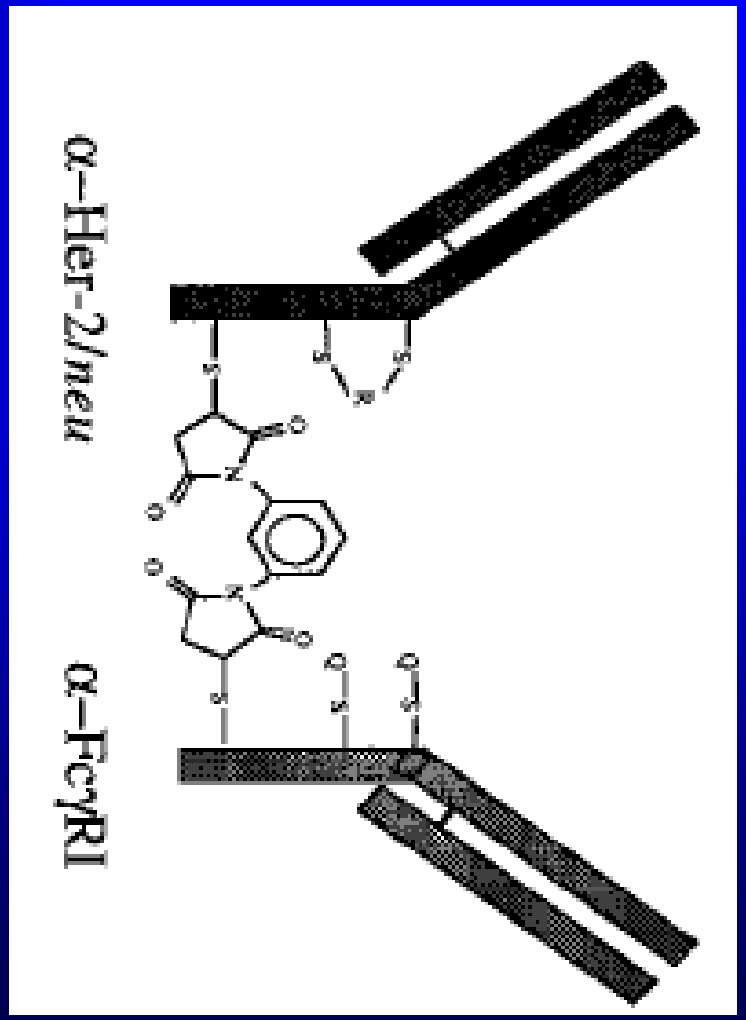
Simultaneous measurement of mitochondrial membrane potential using DiIC1(5) and propidium iodide uptake in ara-C-treated AML cells. Note that the loss of mitochondrial membrane potential occurs prior to the loss of surface membrane integrity (region B).

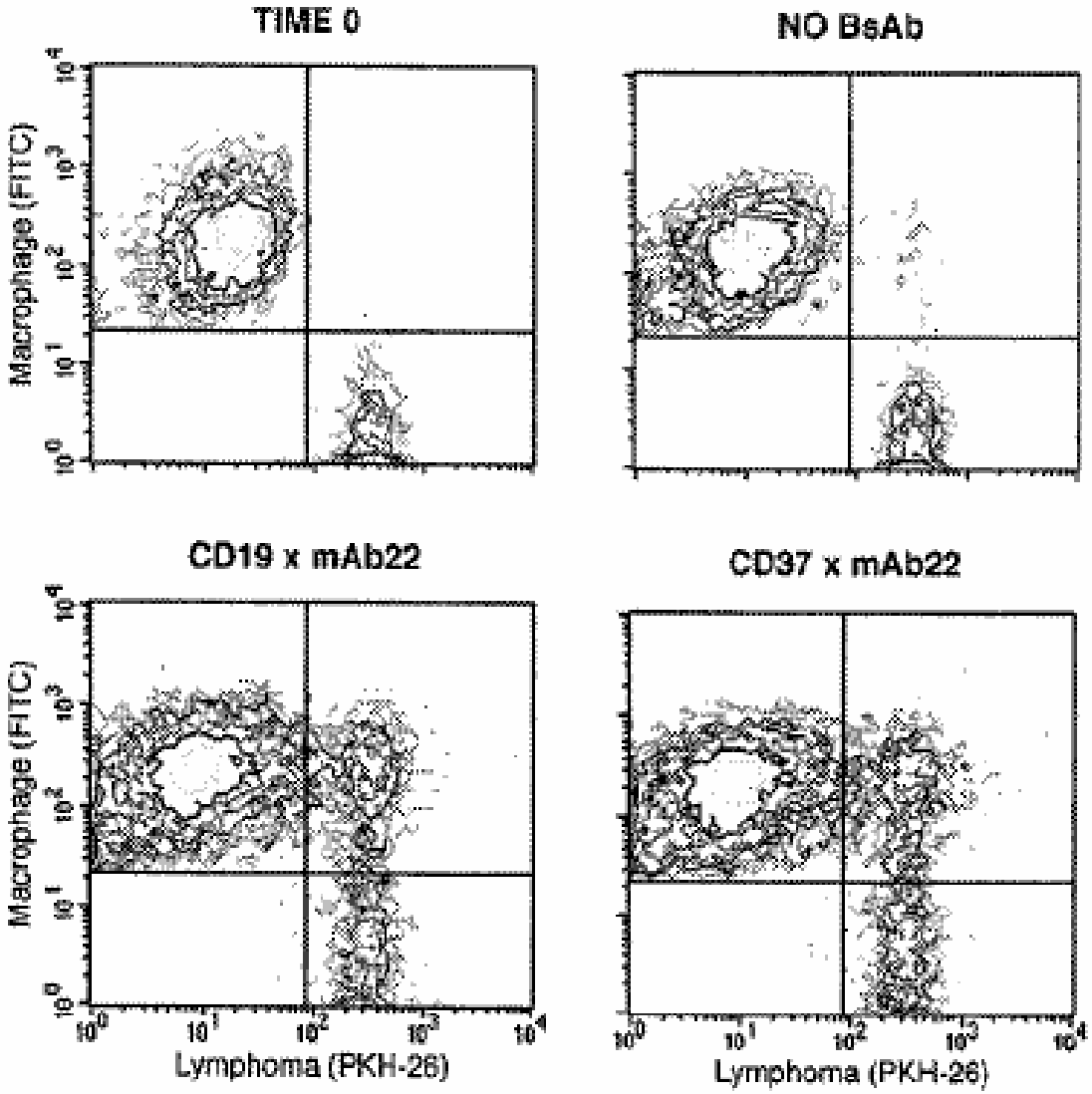


FACS Application

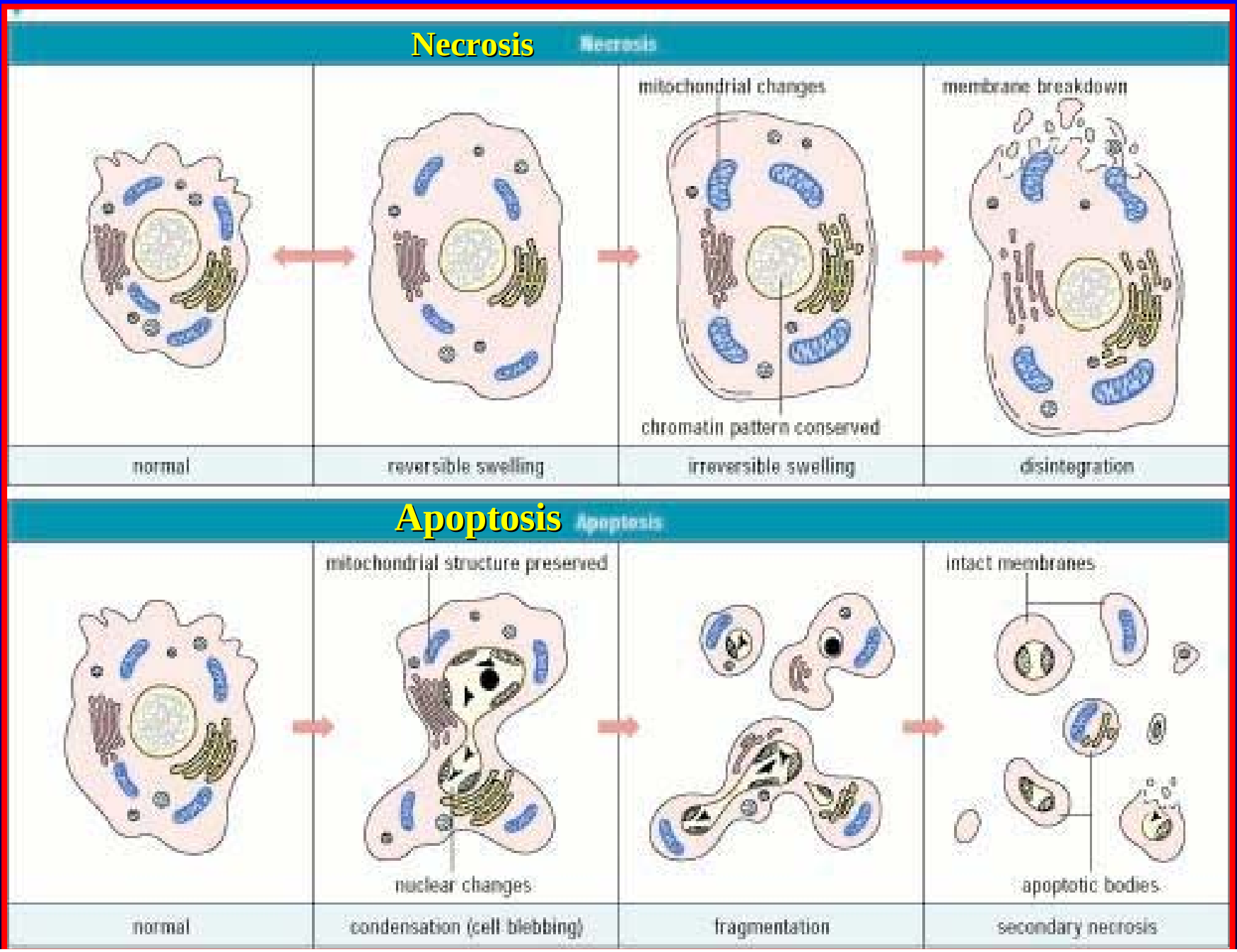


Phagocytosis

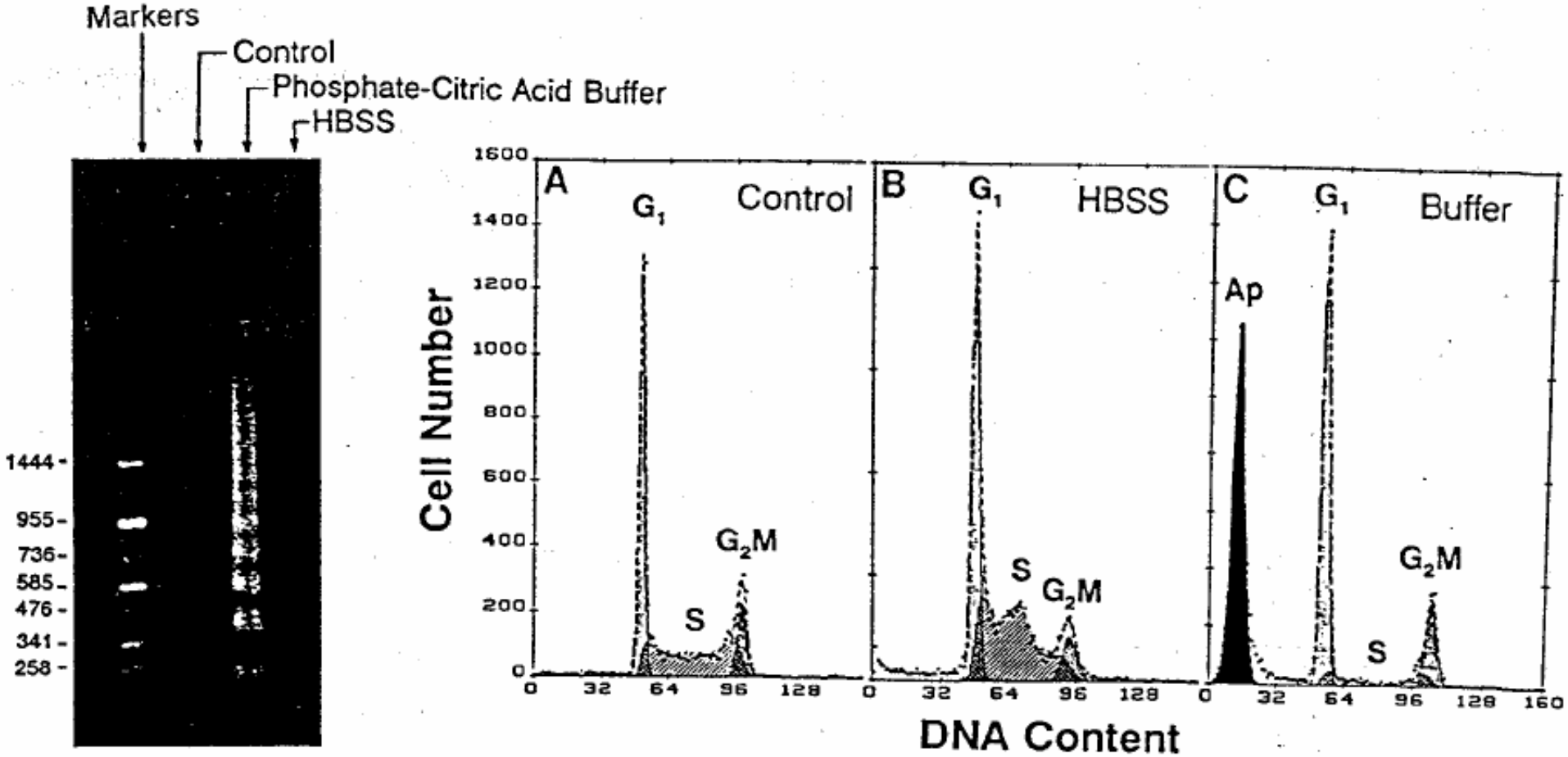




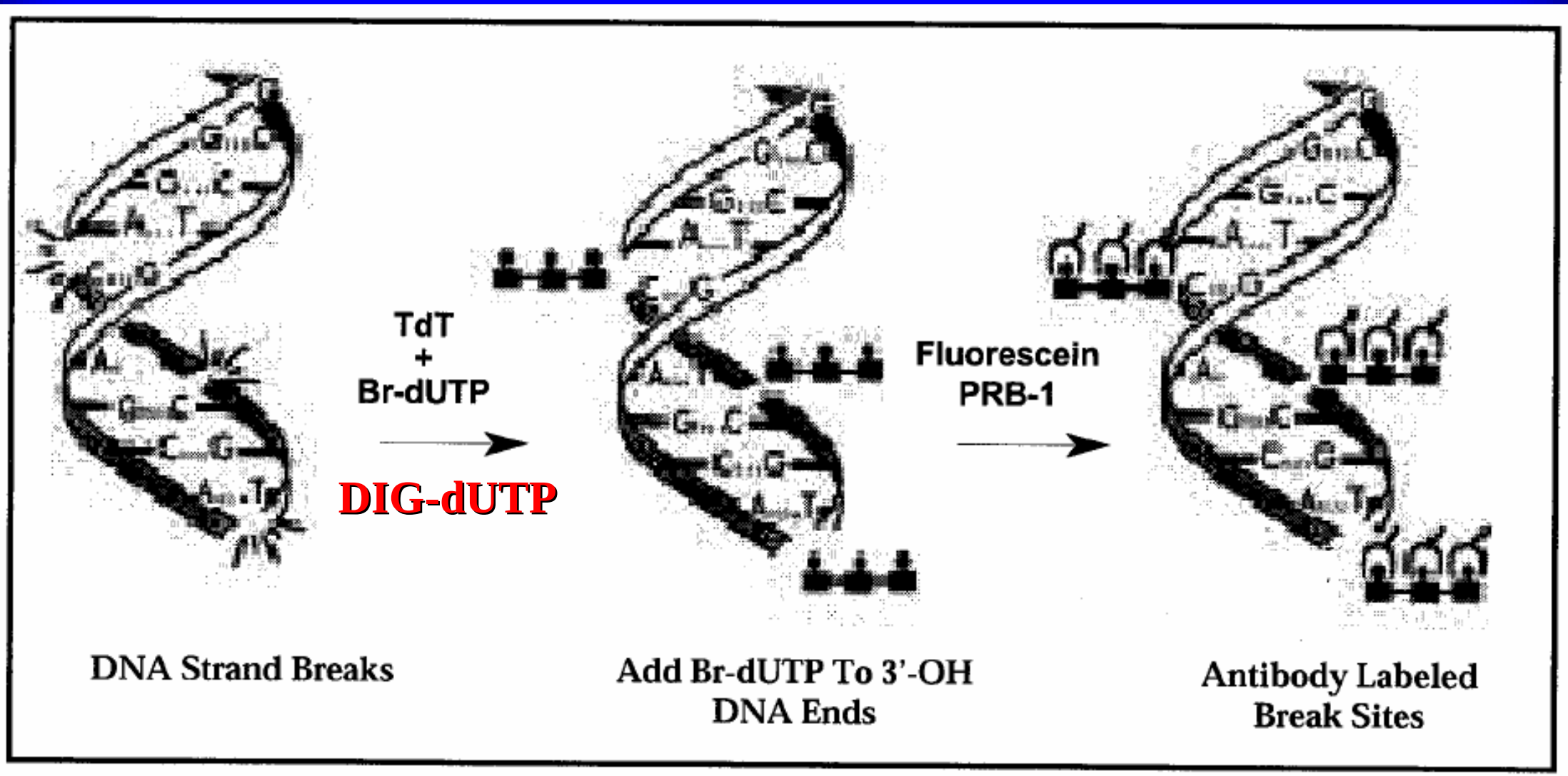
Cell Death


Necrosis
Necrosis
mitochondrial changes
membrane breakdown
chromatin pattern conserved
normal
reversible swelling
irreversible swelling
disintegration
Apoptosis
Apoptosis
mitochondrial structure preserved
intact membranes
nuclear changes
apoptotic bodies
normal
condensation (cell blebbing)
fragmentation
secondary necrosis

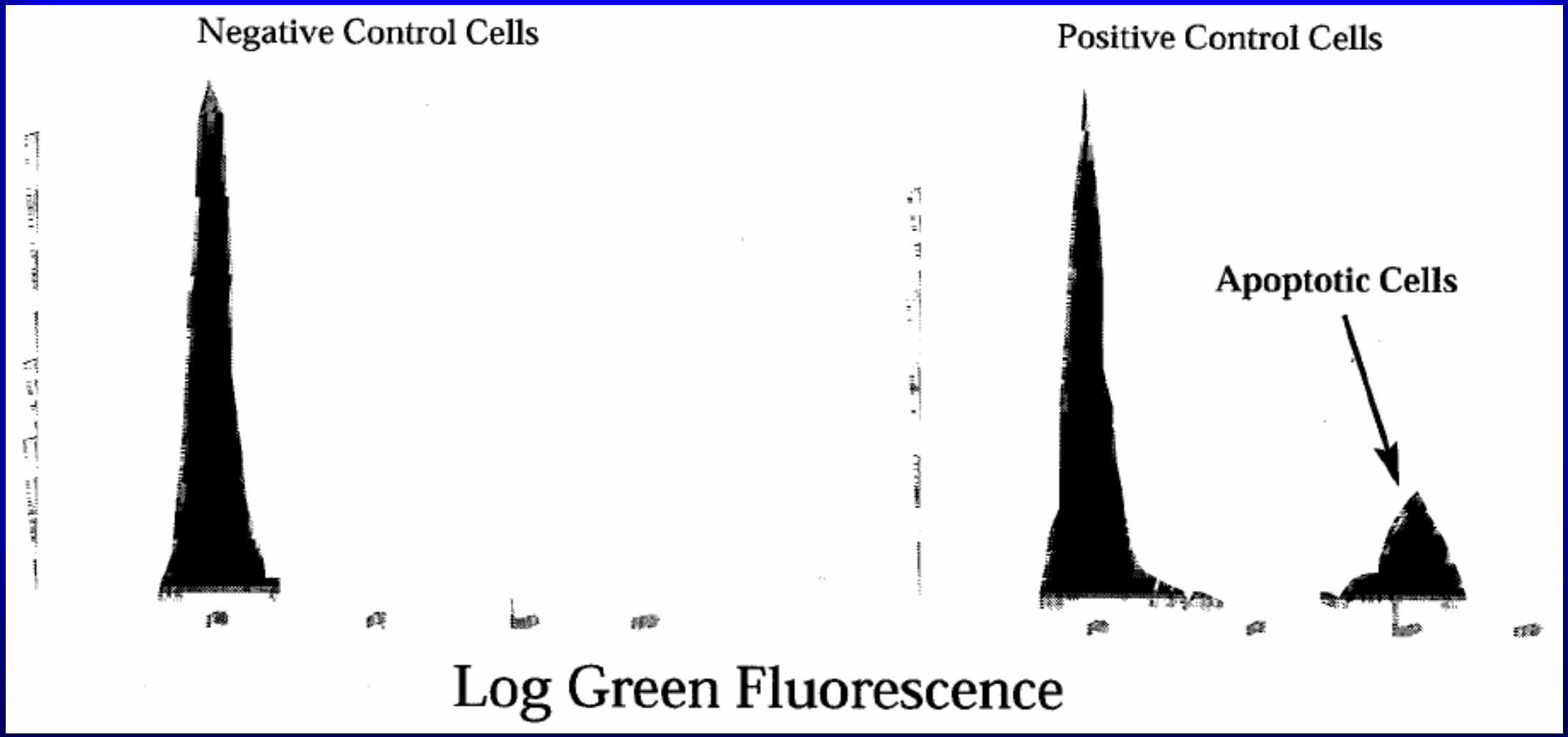
Apoptosis I


Markers
Control
Phosphate-Citric Acid Buffer
HBSS
1444
955
736
585
476
341
258
Cell Number
A
Control
B
HBSS
C
Buffer
G1
S
G2M
Ap
DNA Content

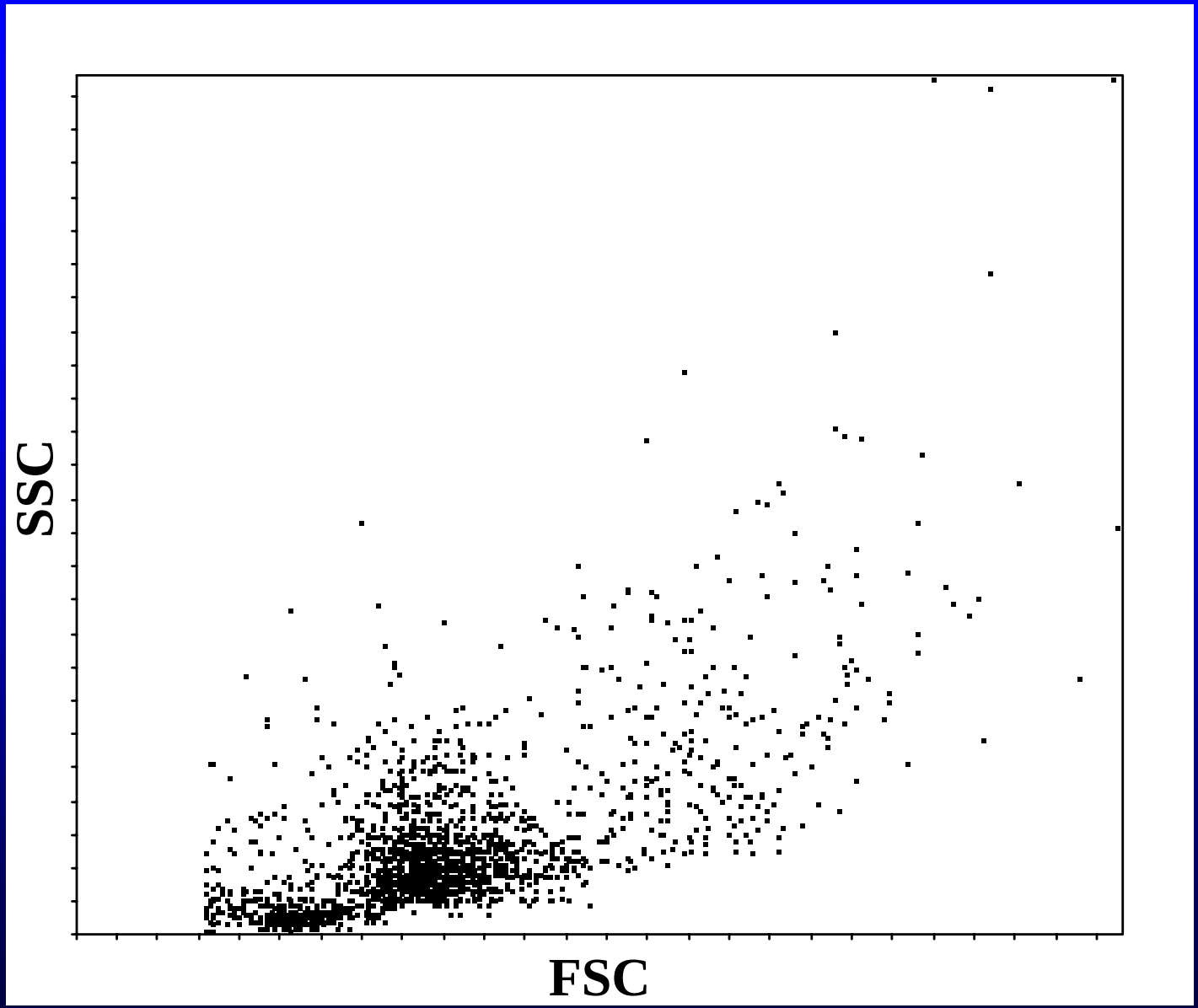
Apoptosis II



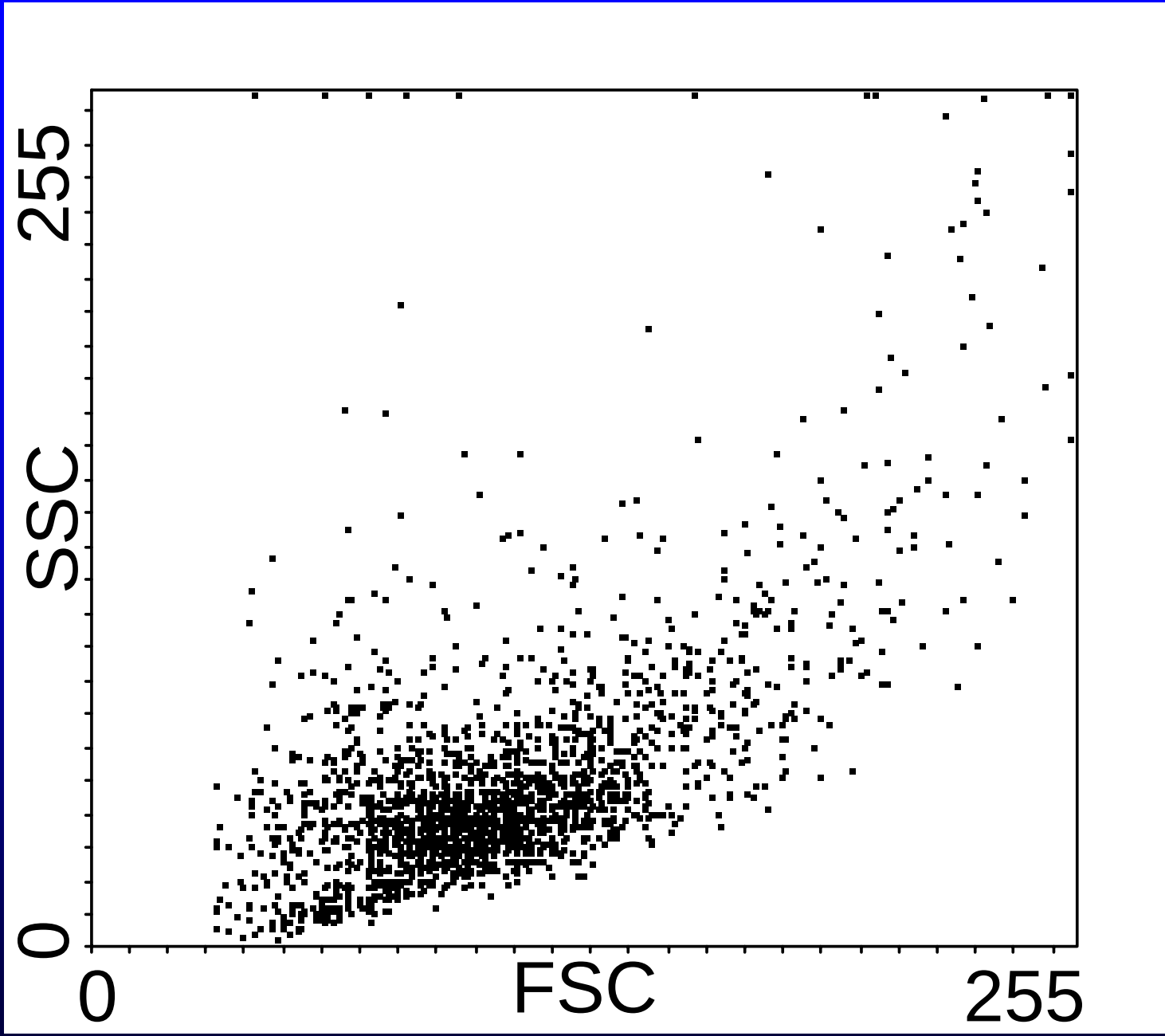
Apoptosis III



Spleen



Tumor : Dot Plot



Tumor Kinetics

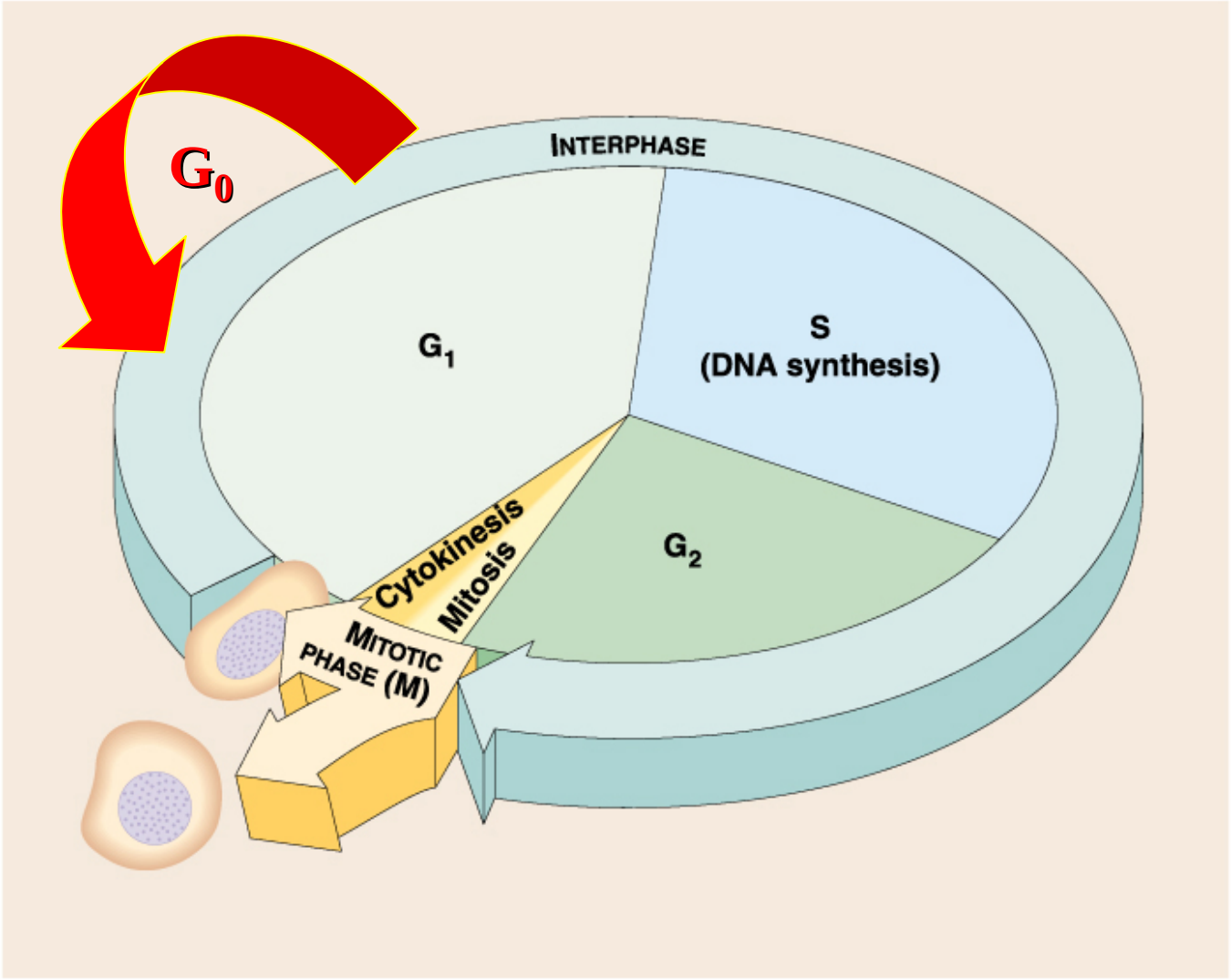
Potential Doubling Time (Tpot)

Growth Fraction (G. F.)
= P / (P + Q)
P: Proliferation cells
Q: Non-Proliferating quiescent cells

if G.F. = 1

Tpot = Tc = λ Ts/L.I.,

or Tpot = Tc/G.F.

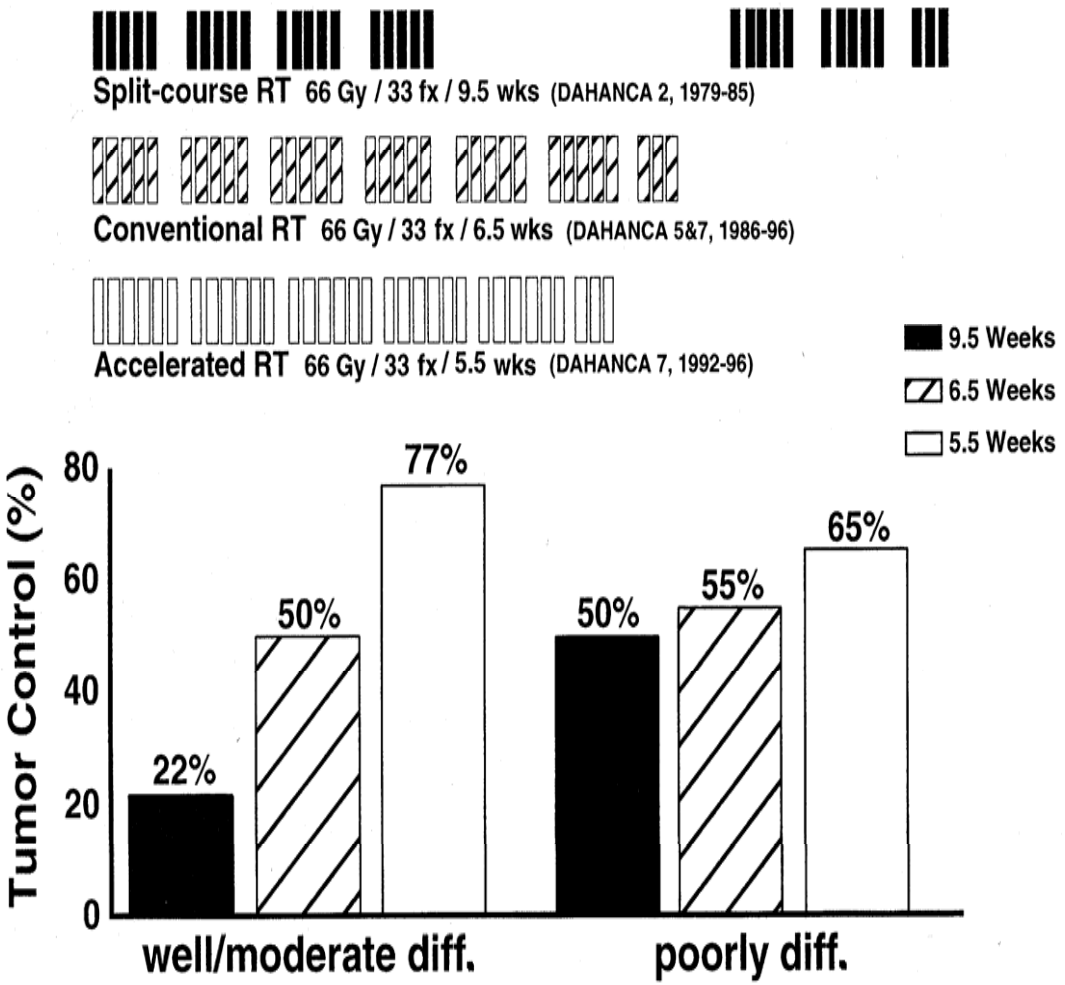


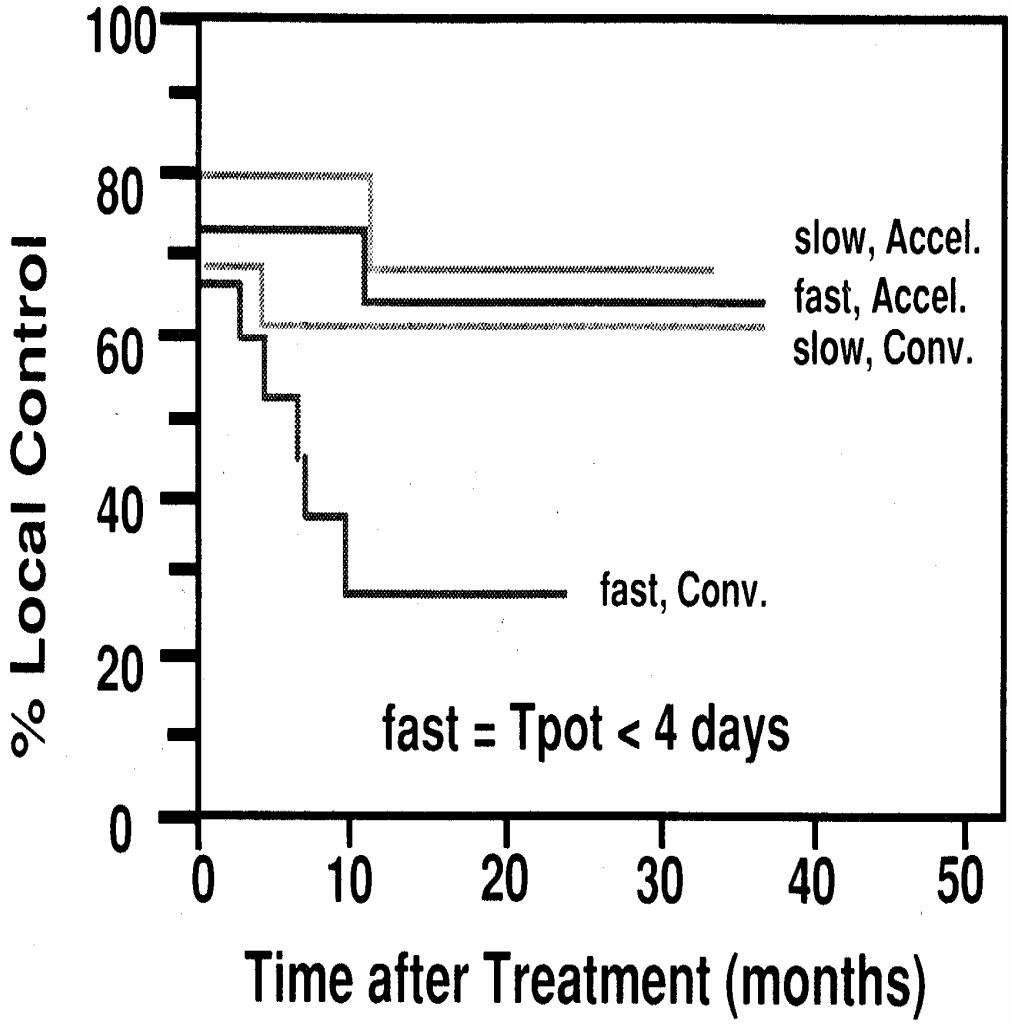
Growth Fraction

TABLE 21.2. *Growth Fraction for Some Tumors in Experimental Animals*

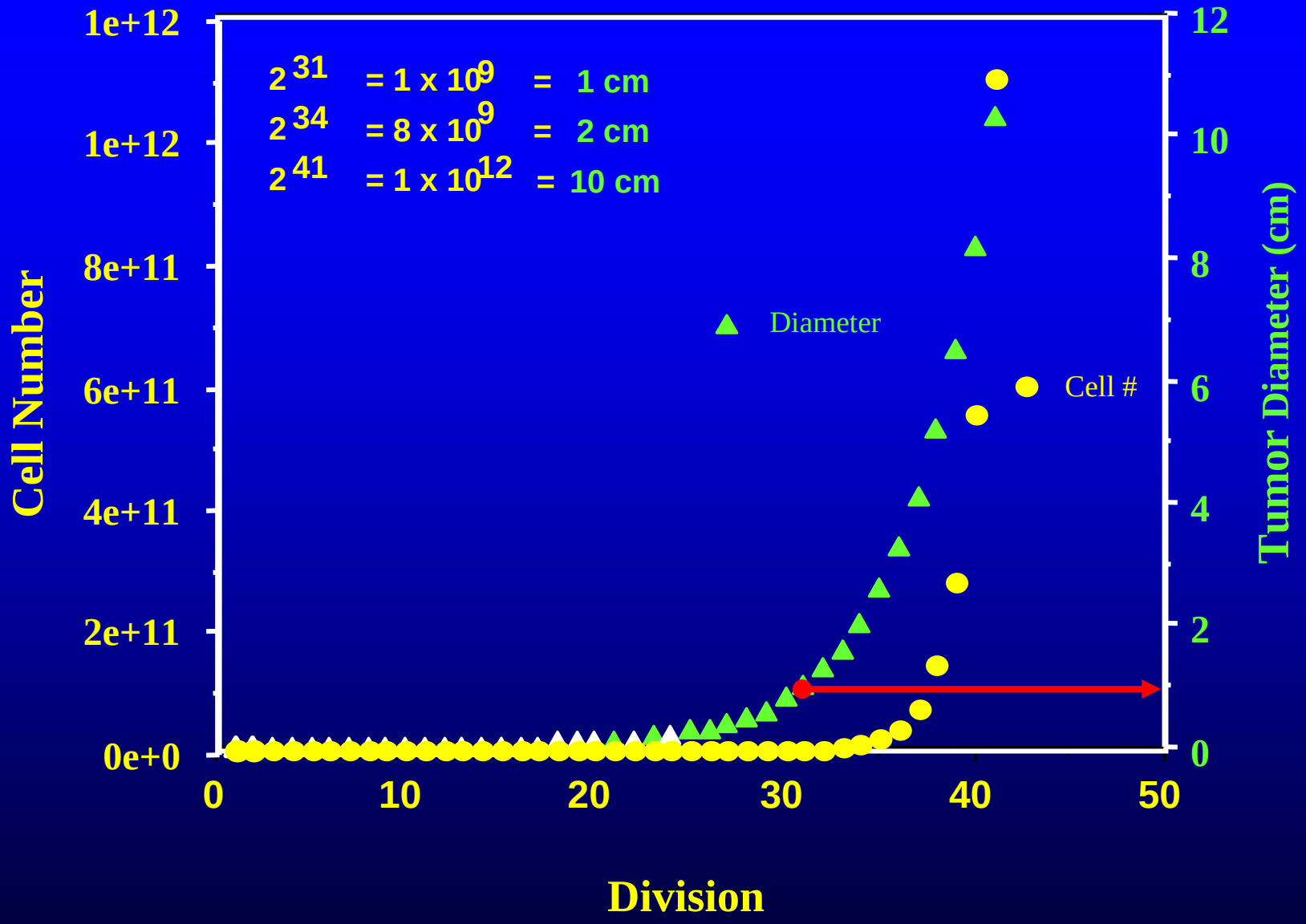
Tumor	Author	Growth Fraction, %
Primary mammary carcinoma in the mouse (G_3H)	Mendelsohn	35–77
Transplantable sarcoma in the rat (RIB_5)	Denekamp	55
Transplantable sarcoma in the rat (SSO)	Denekamp	47
Transplantable sarcoma in the rat (SSB_1)	Denekamp	39
Mammary carcinoma in the mouse (C_3H)	Denekamp	30
Chemically induced carcinoma in the hamster cheek pouch	Brown	29

Clinical Results: Role of T_{pot} in Radiotherapy





Kinetic of Theoretical Tumor Growth



Cell Loss Factor (Φ)

Cell loss factor (Φ)

= 1 – Tpot/Td

Td: observed tumor doubling time

Factors Result in Cell Lost

- Cell Death
- Metastasis
- Exfoliation

Cell Loss Factor (Φ)

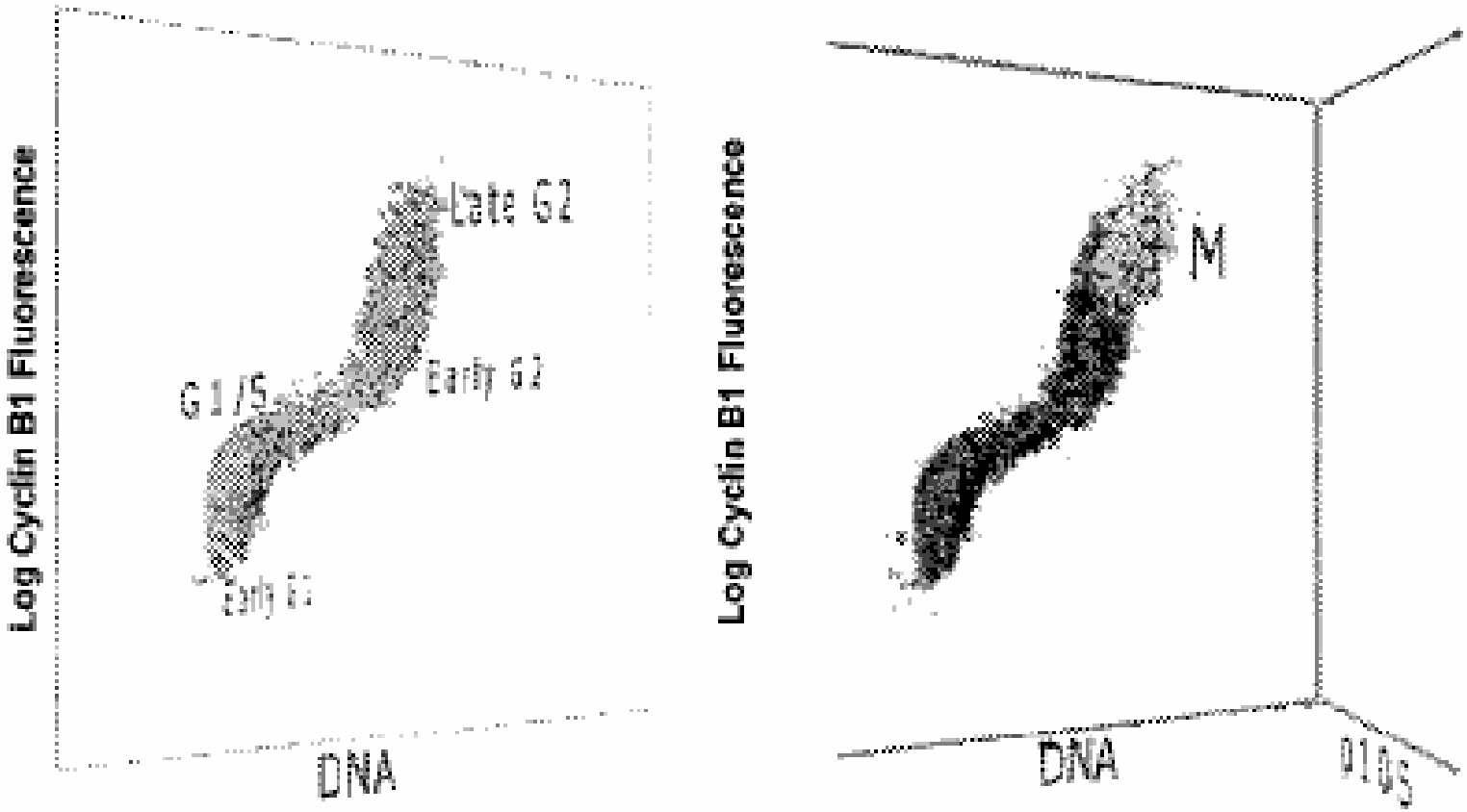
TABLE 21.3. *The Cell Loss Factor (Φ) for Some Tumors in Experimental Animals*

Tumor	Author	Φ, %
Mouse sarcoma	Frindel	
3-day-old tumor		0
7-day-old tumor		10
20-day-old tumor		55
Rat carcinoma	Steel	9
Rat sarcoma	Steel	0
Mouse carcinoma	Mendelsohn	69
Hamster carcinoma	Brown	75
Rat sarcoma	Hermens	26
Hamster carcinoma	Reiskin	81–93
Mouse carcinoma	Tannock	70–92

Roles of Cell Loss Factor in Radiotherapy


http://mx.nthu.edu.tw/~cschiang

Cell Cycle: Marker Protein I



Cell Cycle: Marker Protein II

Table 2. Mitotic Marker Analysis

Antigen	M Phase			
	Prophase	Metaphase	Anaphase	Telophase
H3P	+	+	+	+
Cyclin A	+	–	–	–
Cyclin B1	+	+	–	–
CDK1-P (Thr 161)	+	+	+	–

"+", expressed; "–", unexpressed.

Tumor Control Probability (Chap. 3 page 46)

- **TCP: Probability of no cell survival at all**
- **Poisson statistics :**

$$TCP = e^{-M \times SF} = e^{-(\text{number of cell left})}$$

where M = total number of cells.

- **For $SF = e^{-kD}$ (e.g. single-hit killing)**

$$TCP = e^{-M \cdot SF} = e^{-M \cdot e^{-kD}} = e^{-e^{-(kD - \ln M)}}$$

- **A sigmoid curve**
- **NTCP: Normal tissue complication probability**

Calculation of tumor cell kill (p.46)

1. A tumor consists of 10^9 clonogenic cells. The effective dose-response curve, given in daily dose fractions of 2 Gy, has no shoulder and a D_0 of 3 Gy. What total dose is required to give a 90% chance of tumor cure?

A:

$$TCP = e^{-MxSF}$$

$$90\% = e^{-(10^9)xSF}$$

$$SF \cong 10^{-10} = e^{-aD} = e^{-D/eDo} = e^{-D/3Gy}$$

$$D = 3Gy\ x \ln 10^{-10} = 69\ Gy$$

Poisson Statistical Distribution

- **Probability of r number of drops in the bucket:**

$$P(r) = \frac{e^{-m} m^{r}}{r!}$$

  - For 0 drop, r = 0, P (0) = e^{-m}
  - For 1 drop, r = 1, P (1) = m e^{-m}
  - and so on ...
  - Probability of "no hit at all" (i.e. survival fraction) = P (r = 0) = e^{-m}

TCP vs NTCP