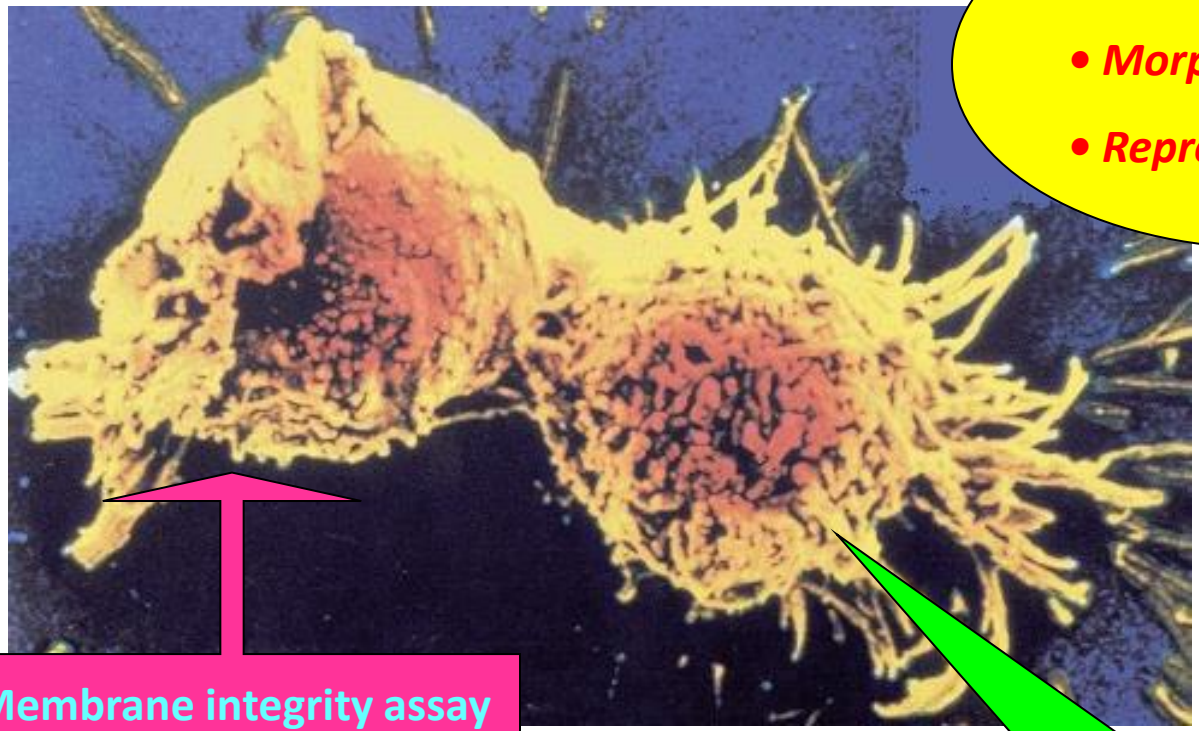
Cell Viability II



- *DNA labeling assay*
- *Morphological assay*
- *Reproductive assay*

Membrane integrity assay

Functional assay

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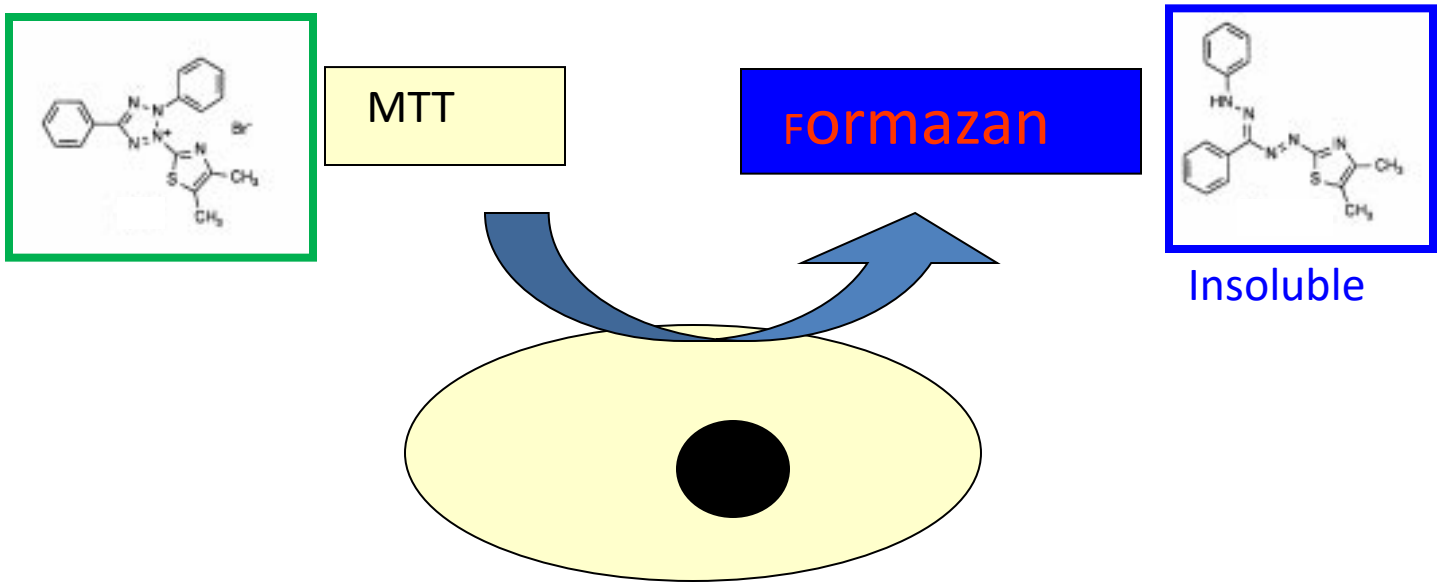
MTT Assay

Introduction

- This assay is a sensitive, quantitative and reliable colorimetric assay that measures viability, proliferation and activation of cells

- The assay is based on the capacity of mitochondrial dehydrogenase enzymes in living cells to convert the yellow water-soluble substrate 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) into a dark blue formazan product which is insoluble in water

- **The amount of formazan produced is directly proportional to the cell number in range of cell lines.**



Materials and equipment

MTT solution (5 mg/mℓ in phosphate buffered saline (PBS) pH 7.5), HCl, Propan-2-ol

96-well microtiter plate, ELISA reader

Procedure (suspension and monolayer cells)

1. **Prepare MTT stock solution and fiter through a 0.2 μm filter to sterilize and remove the small amount of insoluble residue**
2. **To 100 μℓ cell suspension or cell monolayer in each microtiter well add 10 μℓ MTT**
3. **Incubate in a humidified incubator at 37℃ for 3 h**
4. **Add 100 μℓ 0.04 M HCl in propan-2-ol to each well and mix thoroughly to dissolve insoluble dark blue formazan crystals**
5. **Read plate on a ELISA reader using a test wavelength of 570 nm and reference wavelength of 630 nm**

Procedure (immobilized cells)

1. Prepare a solution of MTT in PBS at a concentration of 1 ㎎/㎖. Add 1 ㎖ MTT solution to 0.5 ㎖ immobilized matrices (e.g. beads)
2. Incubate a 37℃ for 3 h to allow MTT to diffuse throughout the matrices and react with cell
3. Centrifuge at 180 g for 2 min.
4. Measure optical absorbance at 570 nm. It may be convenient to dilute

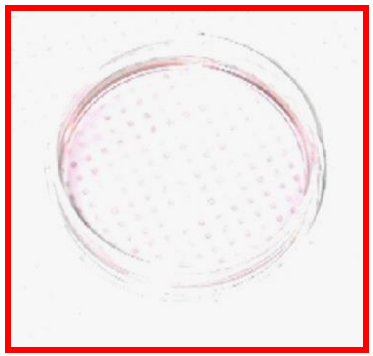
Advantages of MTT assay

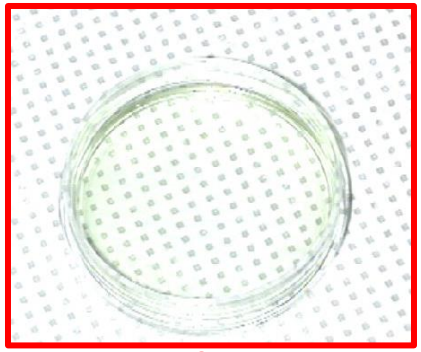
1. Considered a major advance; used the most prevalent in vitro assay
2. Rapid, versatile, quantitative and highly reproducible
3. Adaptable to large-scale screening; relevant for most cells
4. MTT reduction correlates to indices of cellular protein and earlier cell number
5. More sensitive and earlier predictor of toxicity than classical LDH or neutral red measurements

Disadvantage of MTT assay

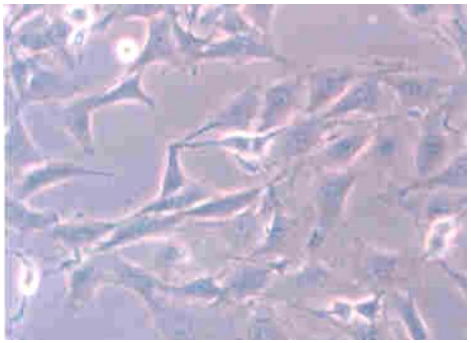
1. Production of the MTT product is dependent on the MTT concentration in the medium. The kinetics and degree of saturation are dependent on cell type
2. Assay is less effective in the absence of cell proliferation
3. MTT cannot distinguish between **cytostatic** (inhibition of cell growth) and **cytocidal** (Causing cell death) effect
4. Individual cell numbers are not quantitated and results are expressed as a percentage of control absorbance
5. Test is less effective if cells have been cultured in the same media that has supported growth for a few day, which leads to underestimation of control and untreated samples

Example: NIH J2 3T3 cell

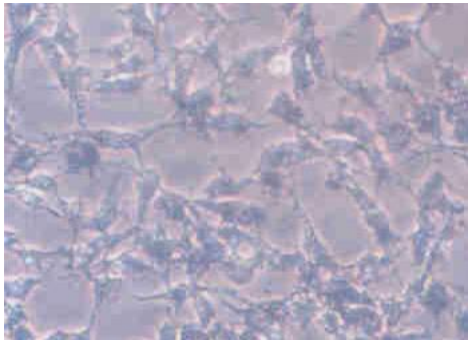




MTT solution



in medium



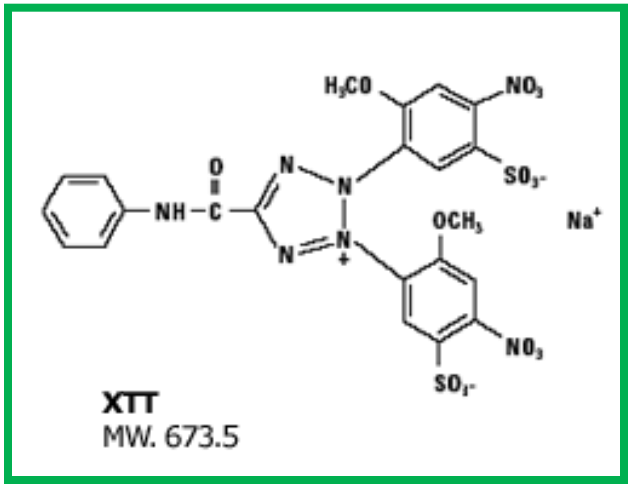
elution

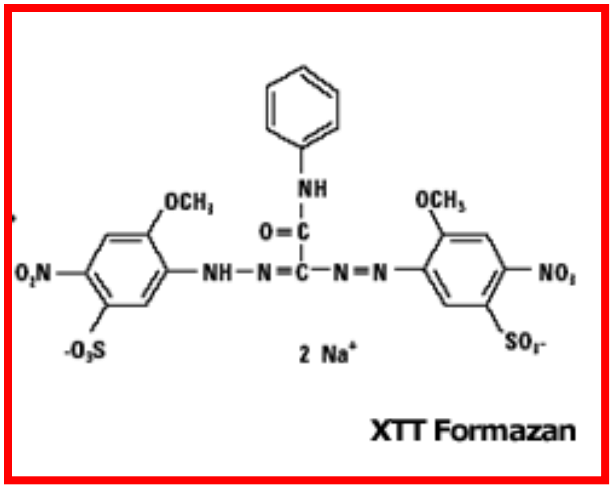
Absorbance at 570 nm

XTT assay

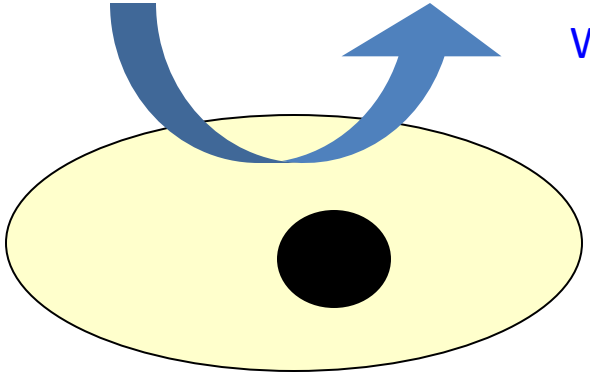
Introduction

- The assay is based on the cleavage of the yellow tetrazolium salt XTT to form an orange formazan dye metabolic active cells
- This conversion only occurs in viable cells
- The formazan dye formed is soluble in aqueus solution and is directly quantified using Microplate reader
- Both MTT and XTT work by being to a formazan dye only by metabolic active cells





Water soluble



metabolically active cell

Materials and equipments

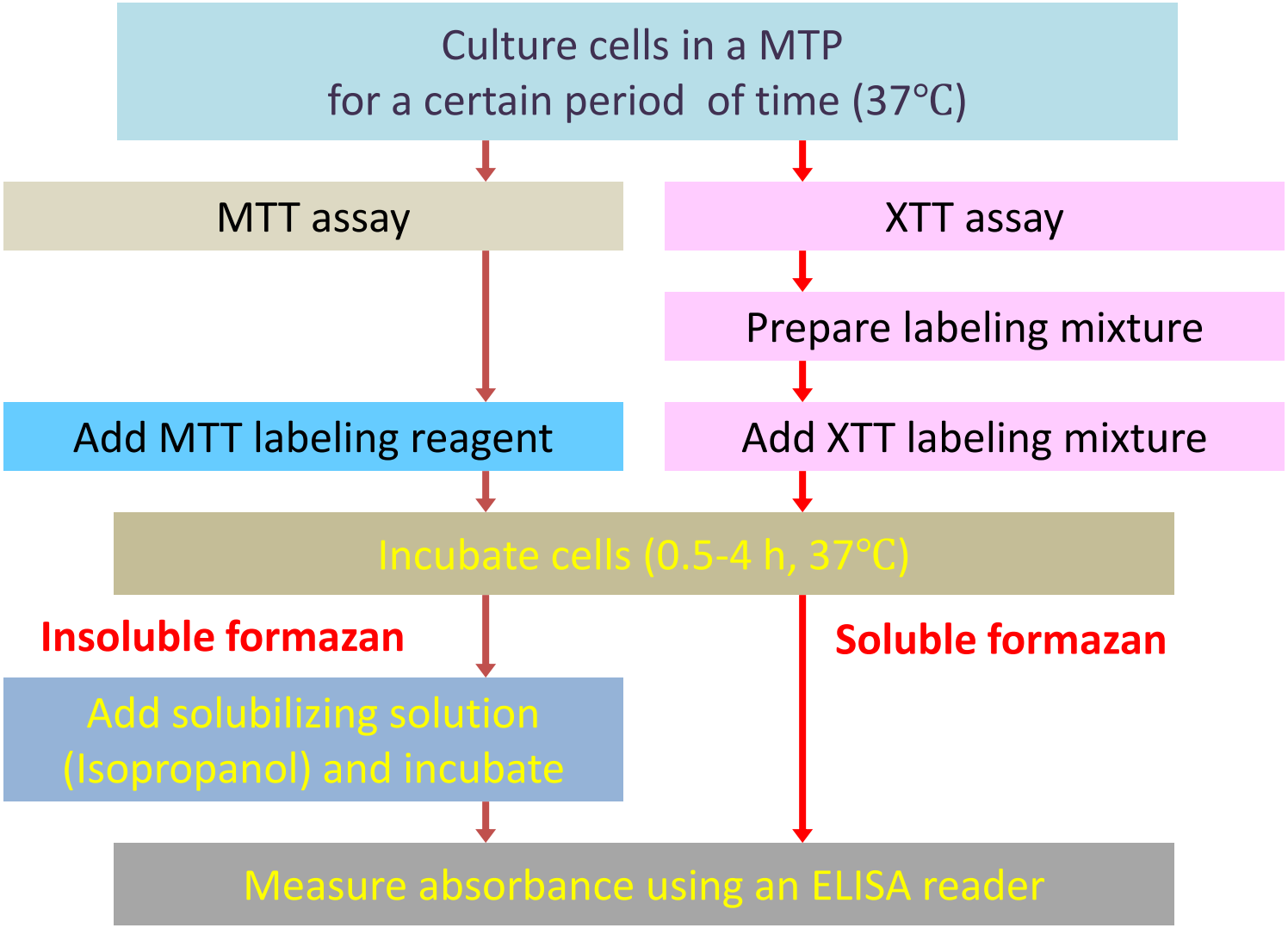
XTT labeling regent, electron-coupling reagent
96-well microtiter plate, Microplate reader

→ XTT labeling mixture : mixed 5 ㎖ XTT labeling reagent with 0.1 ㎖ electron coupling reagent

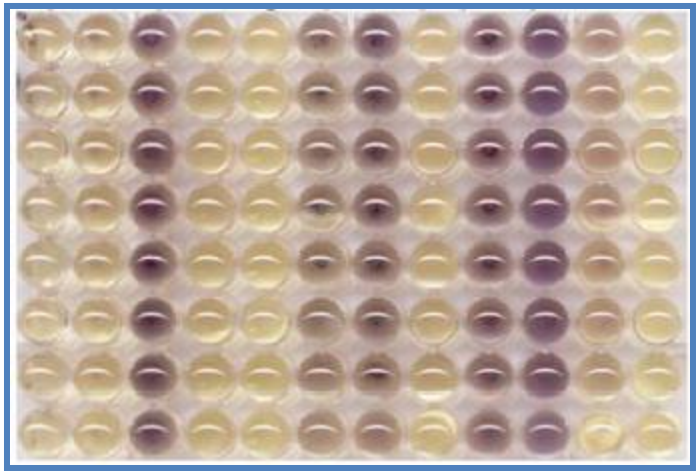
Procedure

1. Cell are grown in microtiter plates in a final volume of 100 ㎕ culture medium per well. The incubation period of the cell cultures depends on the particular experimental approach and on the cell line
2. After incubation period, add to each well 50 ㎕ of the XTT labeling mixture
3. Incubate the microtiter plate for 4 to 24 h in incubator
4. Read plate on a Microplate reader using a wavelength between 450 and 500 nm (reference wavelength of 650 nm)

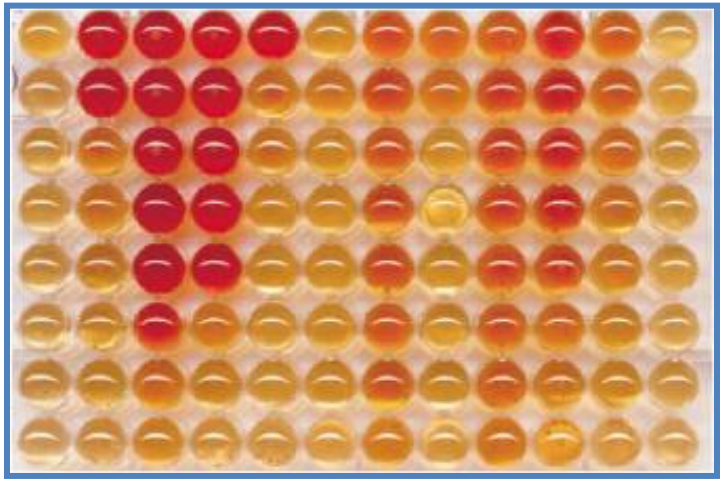
Compare with MTT assay and XTT assay



Compare with MTT assay and XTT assay



MTT



XTT

Jenny G., Mark H., Anna J., Inger K., Douglas Mc., Roland M., 2002.
Evaluation of redox indicators and the use of digital scanners and spectrophotormeter for quantification of microbial growth in microplates. J. Micro. Methods. 50:63-73

Crystal violet

Principle

- **Dye elution:**

  Cell up-taken dye was measured colorimetric method after acetic acid dye elution

- **Nuclei counting**

  Incubation of cell samples in a mixture of citric acid and crystal violet causes cells to lyse and the released nuclei to stain purple

Procedure

❖ Dye elution

① After removal of medium, rinse 96 well plates with 100 μℓ/well of PBS and stain with 100 μℓ 0.25% (g/10ml) aqueous crystal violet for 10 min.

② Rinse plats four times in tap water.

③ Dry the outsides of the plates with paper to help avoid water stains, and then dry the plates at 37°C. When dry, add 100 μℓ per well of 33% glacial acetic acid (33 ml/100ml) and mix the contents of each well before reading at 570 nm.

❖ Nuclei counting

① Allow microcarriers from a culture sample (1ml) to settle to the bottom of a centrifuge tube.

② Removed clear supernatant by aspiration.

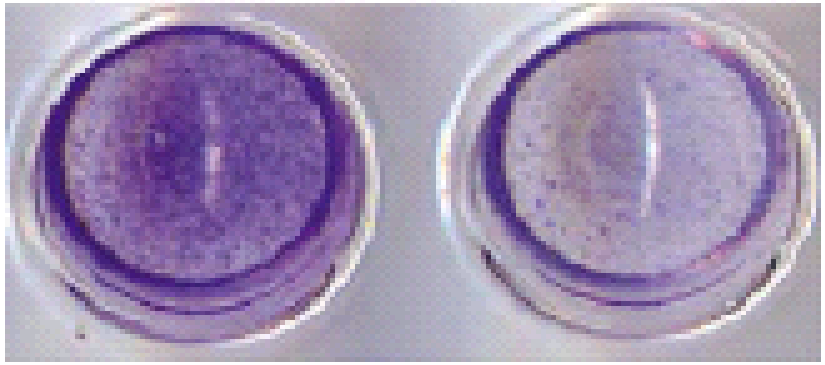
③ Add 1ml of crystal violet reagent.

④ Incubate at 37°C at least 1 h.

⑤ Introduce a sample into the hemocytometer chamber and count the purple-stained nuclei as for whole cells.

Example; Monolayer culture

Crystal Violet - 60minutes

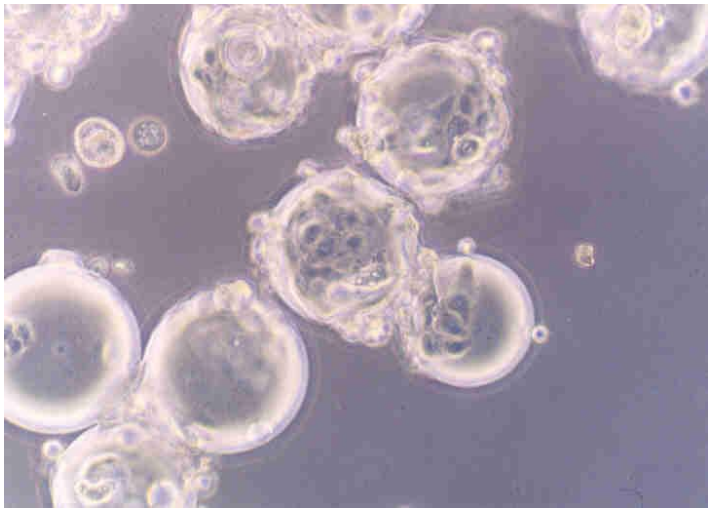


Non-OP **OP**

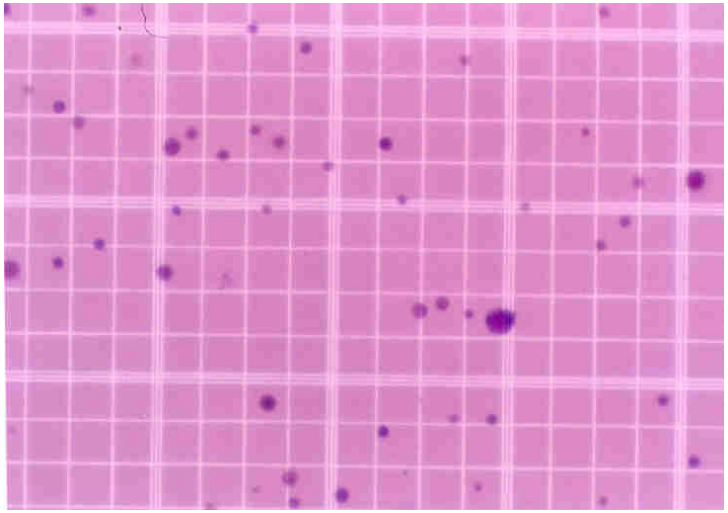
※ OP : Osteoporosis

Ref.:Journal of Orthopaedic Resarch19 (2001)

Example; Microcarrier culture



Rabbit oral mucosal cell cultured by microcarrier



Cell counting

Acid phosphatase (AP) assay

Introduction

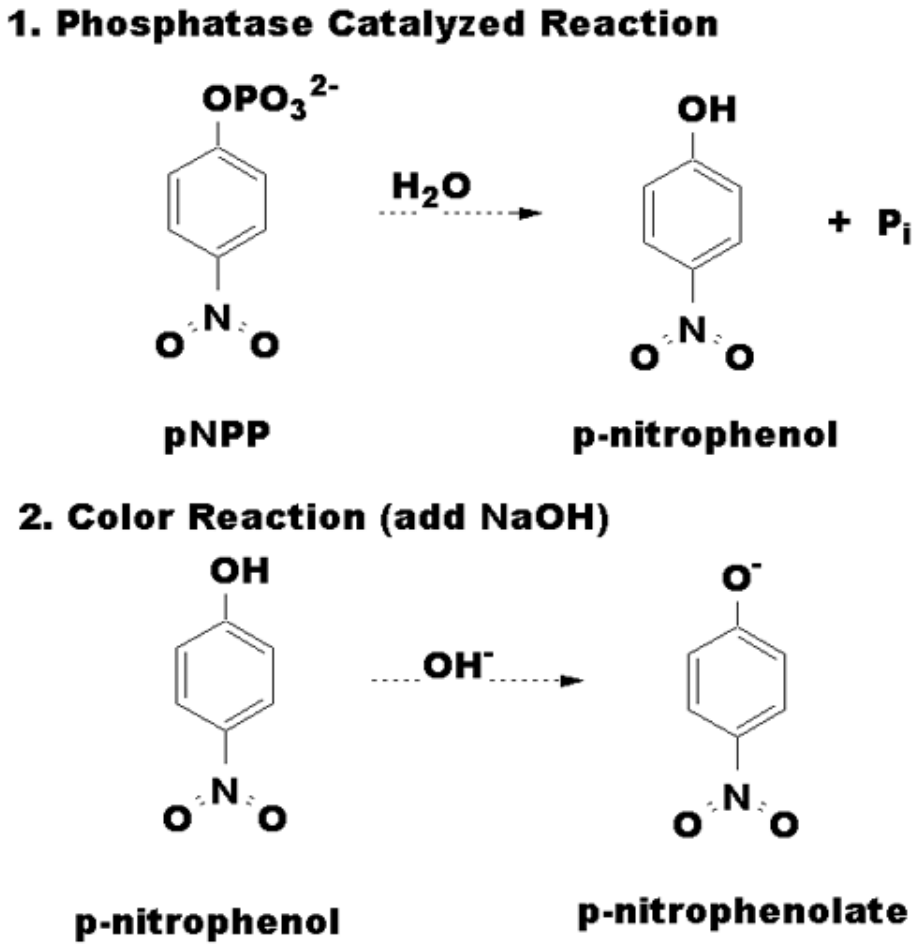
- The action of this enzyme in many of tissue is to cleave a waste product called pyrophosphate and effectively convert it to a useable phosphate

- P-nitrophenyl phosphate will be the substrate and nitrophenol is the product of this reaction

- Nitrophenol is colorless but when the pH of the reaction solution is alkaline, it is appears yellow. The pH of the reaction solution will be changed by the addition of NaOH

P-nitrophenyl phosphate + Acid phosphatase

→ Nitrophenol + HPO_4^{-2}



Materials and equipment

Substrate-containing buffer : 10 mM P-nitrophenyl phosphate in 0.1 M sodium acetate pH 5.5, 1 M NaOH

96-well micro titer plate, Microplate reader

Procedure

1. **At end of cell growth period, remove medium and rinse wells in 100 µℓ PBS**
2. **Add 100 µℓ substrate-containing buffer to each well**
3. **Incubate for 2 h in incubator. Read plates at 405 nm, and either reincubate for a further time if increased sensitivity is required, or 'stop' with addition of 50 µℓ/well of 1 M NaOH to cause an electrophilic shift in the p-nitrophenol chromophore and thus develop the yellow color, giving greatly increased sensitivity**

Alamar Blue oxidation-reduction assay

- **Principle**

  In the presence of cellular metabolism the color of Alamar Blue (ALB) changes from a fully oxidized, non fluorescent blue to a fully reduced, fluorescent red. ALB will be reduced by a variety of enzymes and small molecules, including the cytochrome system, FMN, FAD, NAD, and NADP

- **Advantages**

  Simple, rapid, inexpensive, required no lysis, extraction or washing of sample

- **Disadvantages**

  Unstable during storage (absorbance of oxidized ALB-3day, reduced ALB-increase from one day to the next)

- **Characteristics**

  - Sensitivity : Propidium iodide (PI), Sulforhodamine B (SRB) > ALB=MTT

  - The ALB assay is faster, simpler, and less artefact prone than the MTT assay

Procedure

① At the end of an experimental incubation period, add 1 vol of ALB stock solution per 25 vols (4%v/v) of growth medium in each well (8 μℓ ALB for 200 μℓ of growth medium)

② Incubate plates at 37℃ for 3 h to allow metabolic dye reduction.

③ Equilibrate plates to room temperature for 30 min in the dark.

④ Measure the relative fluorescence at 530~560 nm excitation and 590 nm emission wavelengths.Fluorescence is temperature sensitive; either equilibrate plates in a warm room at the culture incubation temperature. For better sensitivity, measure the fluorescence in bottom-reading rather than top reading mode. The ratio of test to control fluorescence values at 590nm measures the effect of a treatment on cell growth or metabolism.

⑤ For spectrophotometric assays, correct for the spectral overlap of the oxidized and reduced forms of ALB by measuring each sample at two different wavelengths, between, approximately, 540~630 nm . One of these must be a low wavelength (LW) and the other a high wavelength (HW); for example, 570 ~ 600 nm, respectively.

⑥ A correct factor (RO) for the absorbance of oxidized alb must be calculated.

ⓐ Measure the absorbance (AM) of growth medium alone. (no ALB)

ⓑ Measure the absorbances of oxidized (blue) ALB in growth medium at the low and high wavelengths.

ⓒ Substract AM from each of the measured ALB absorbance to produce, respectively, AOLW and AOHW , the absorbance of oxidized (blue) ALB at the low and high wavelengths.

ⓓ Calculate the correction factor RO of oxidized ALB:
RO=AOLW/AOHW

⑦ Measure the absorbance values (ALW and AHW) of a test sample at each wavelength.

⑧ Calculate the percentage of reduced ALB (ARLW) in a sample as:
ARLW = 100 x [ALW-(AHW x RO)].

⑨ Calculate the percentage difference in reduction (PDR) between treated and control cells: PDR = 100 x (test ARLW/ARLW for positive growth control)

Neutral Red assay

(3-amino-7dimethyl-2-methyphenazine hydrochloride)

❖ **Principle**

- The incorporaton of NR into the lysosomes of viable cells after their incubation with test agents

❖ **Use**

- Industrial, pharmaceutical, environmental and other testing laboratories concerned with acute toxicity testing

❖ **Advantages**

- Simplicity, speed, economy, and sensitivity

Materials and Equipments

❖ Solution

① Neutral red

4mg/ml stock solution

Dilute 1:100 into medium , incubate overnight at 37℃ and centrifuge for 10 min at 1500 g before use.

② 1% $CaCl_2$/0.5% formaldehyde

Mix 6.5 ml 37% formaldehyde with 50 ml 10% $CaCl_2$ and 445 ml distilled water.

③ 1% acetic aicd/50% ethanol

Mix 4.75 ml acetic acid with 250 ml 95% ethanol and 245 ml distilled water.

❖ Equipment

① Complete media suitable for chosen cell type.

② Culture petri dish

③ 96well tissue culture plate

④ Inverted microscope

⑤ ELISA-type spectrophotometer

⑥ Microplate shaker

⑦ Eight-channel pipette

Procedure

① Resuspend cells of actively growing culture and count cells and accurately allocate appropriate number suspended in medium.

② Seed 0.2 ml containing desired number of cells to each well of 96 well plate and incubate at 37℃ for 24 h or longer.

③ Removed the medium and add fresh medium containing graded dilutions of test agent. Incubate for desired length of time. Examine at least 4-8 wells per concentration of test agent.

Keep serum concentration as low as possible during this step.

(prevent or to reduce adsorption of xenobiotic to serum components)

④ After incubation for desired time interval, remove medium with test agent and incubate cells with fresh medium containing 40 μg/ml NR dye.

⑤ Continue incubation for 3h to allow for incorporation of vital dye into survival cells.

⑥ Remove medium by inverting the plate and rapid rinse with a mixture of 1% $CaCl_2$ / 0.5%formaldehyde.

⑦ Extract dye into suprernate with 0.2 ml of solution of 1% acetic acid/50% ethanol.

After10 min at room temperature and rapid agitation for a few seconds on a micrometer plate shaker, scan the plate with an ELISA-type spectrophotometer equipped 540 nm filter.

Example

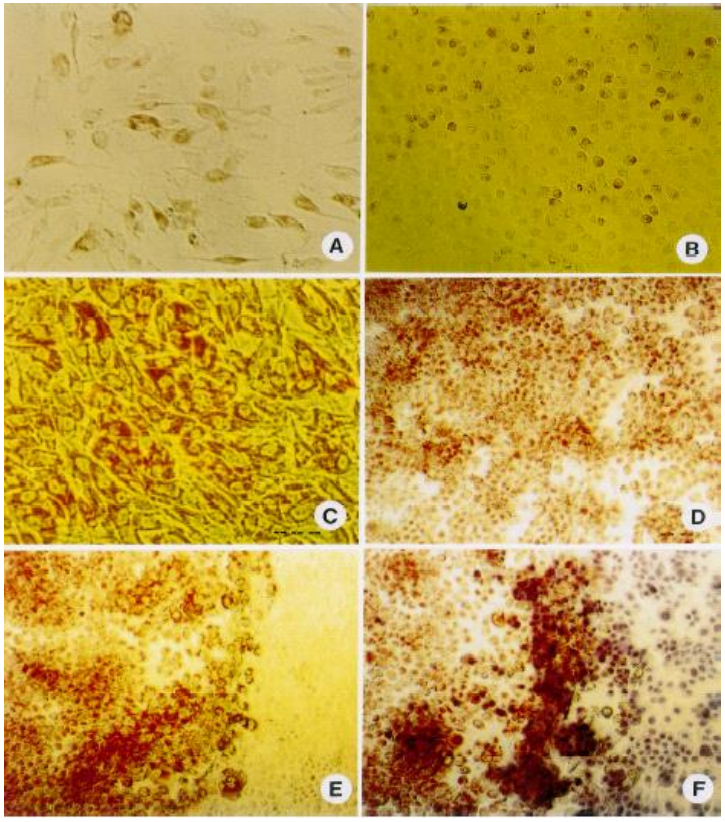


Fig. Monocellular cultures of “fibroblasts and immortalized human gingival keratinocytes after exposure to the materials (dye staining). Cell derangement in the transition area of neutral red destained - and stained cells, indicating spindle- or fusiform cell shape in conventional L-929 mouse fibroblasts (A) and rounded up morphology in immortalized human gingival keratinocytes (B) after methyl methacrylate/monomer incubation.

Confluent cultures of L-929 cells (C) and keratinocytes (D) exposed to Orthocryl Clear. In both cultures, neutral red remains incorporated in the membrane. Neutral red-fading in the transition zone of an inhibition area after Durabase exposure (E) and the cell damage emphasized by trypan blue counterstaining (F). Scale bars are 55 lm (A, C) and 90 lm (B, D, E, F).

Ref.:Biomaterials 21 (2000) 1549~1559

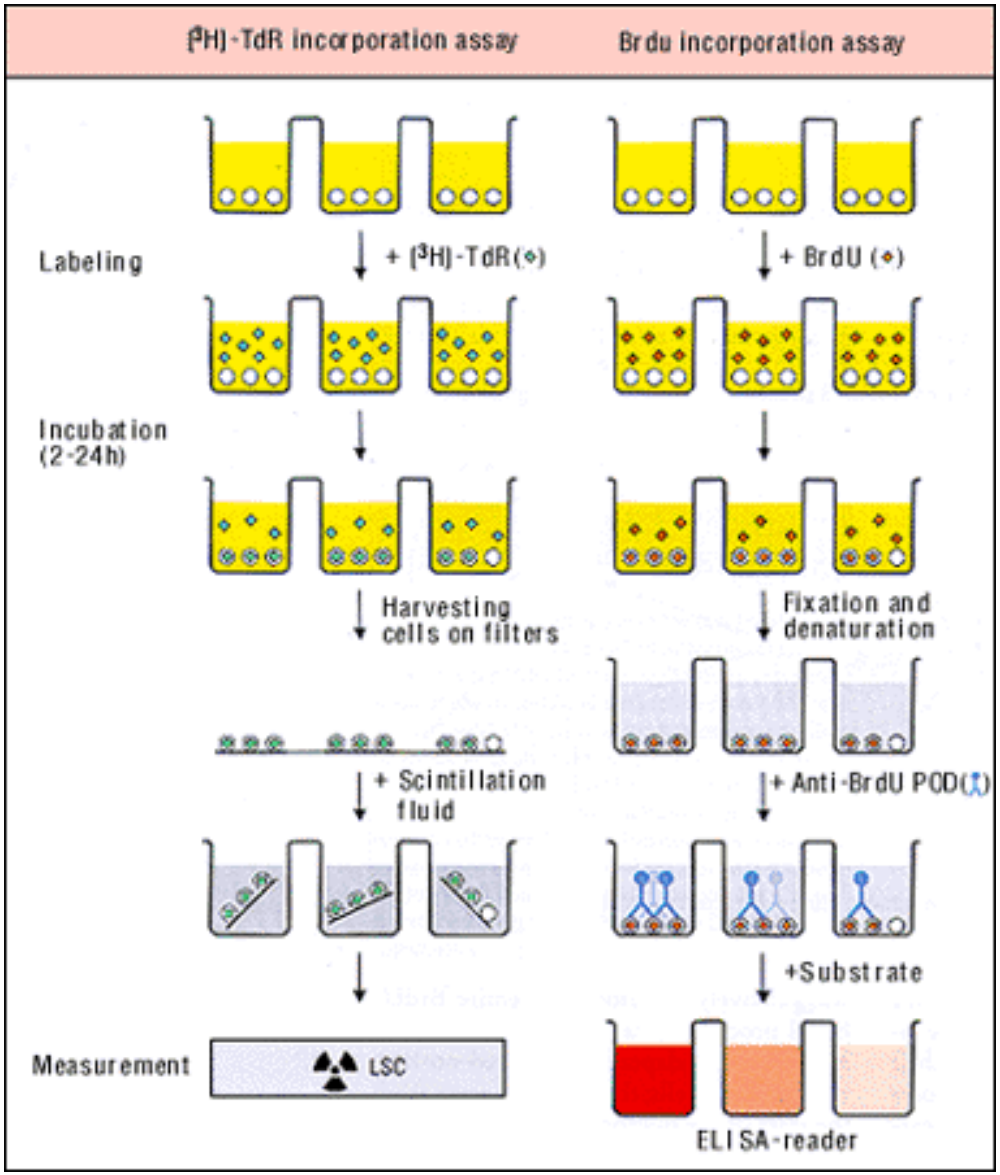
[^{3}H]-thymidine and BrdU incorporation

(DNA synthesis measurement)

Principle

The rate of DNA synthesis is a reflection of proliferation under many condition. To measure the proliferative rates by [^{3}H]-thymidine uptake, cells are cultured in microtitre wells, thymidine is added, and the uptake by DNA is measured , after lysing and washing on, by scintillation counting. Bromodeoxyuridine(BrdU) can be incorporated instead of [^{3}H]-thymidine and the incorporation can be assayed with antibodies to BrdU in a non-radioactive assay.

Schematic diagram of [^{3}H]-TdR and BrdU



Labeling index with [^{3}H]-thymidine

① **Set up the culture at $2x10^4$ cells/ml~ $5x10^4$ cells/ml in 24 well plates containing cover-slips. Grow to the desired cell density.**

② **Add [^{3}H]-thymidine to the medium . 100KBq/ml(~5μCi/ml)and incubate for the cultures 30 min.**

③ **Remove the labeled medium, and discard it into a designed container for radioactive waste.**

④ **Wash the cover-slips three times with PBSA.**

⑤ **Add 1:1 PBSA: acetic methanol, 1ml per well, and remove it immediately**

⑥ **Add 1ml of acetic methanol at 4 to each well, and leave the cultures for 10min.**

⑦ **remove the cover-slips, and dry them with a fan**

⑧ **mount the cover-slip on a microscope slide with the cells uppermost.**

⑨ **Leave the mountant to dry overnight.**

DNA synthesis by [^{3}H]-thymidine

① Grow the culture to the desired density.

② [^{3}H]-TdR, 40 KBq /ml(~1.0μCi), 2 MBq/mol(~50 μCi/mol) in HBSS.

※1Ci =3.7*10^{10} 회/ s = 3.7*10^{10} Bq , 1Bq =1회/s

③ Incubate the cell for 1-24 h.

④ Remove the radioactive medium carefully.

⑤ Wash the cell carefully with 2 ml of HBSS, PBSA, and 2 ml ice-cold 0.6 M TCA for 10 min.

⑥ Wash the cell with TCA twice 5 min each time.

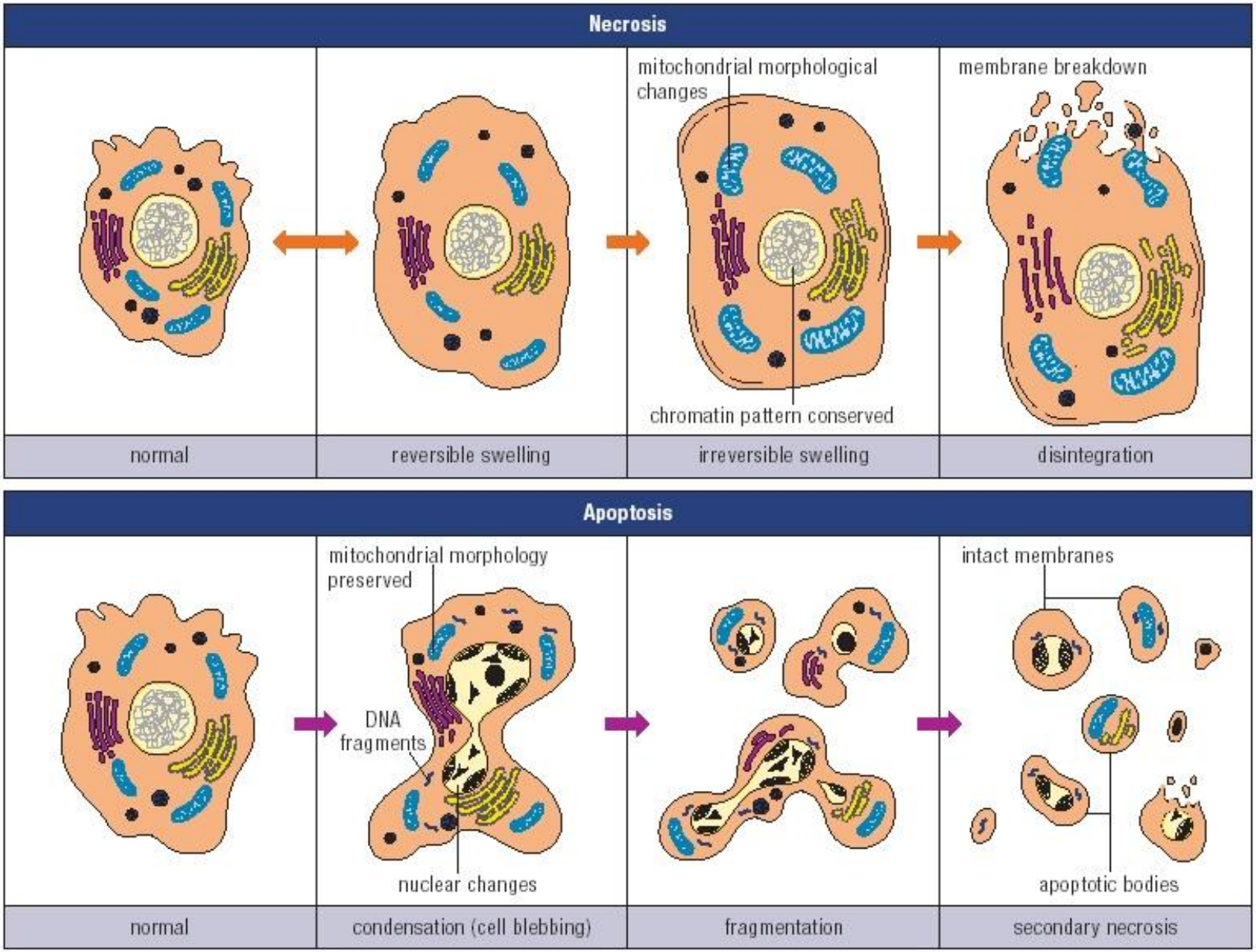
⑦ 0.5 ml of 2 M perchloric acid, a hot plate at 60℃ for 30min and allow the solution to cool.

⑧ Add 0.5ml SLS in NaOH incubate the solution at 37℃ for 30 min or overnight at room temperature.

⑨ Collect the solubilized pellet and determine the radioactivity.

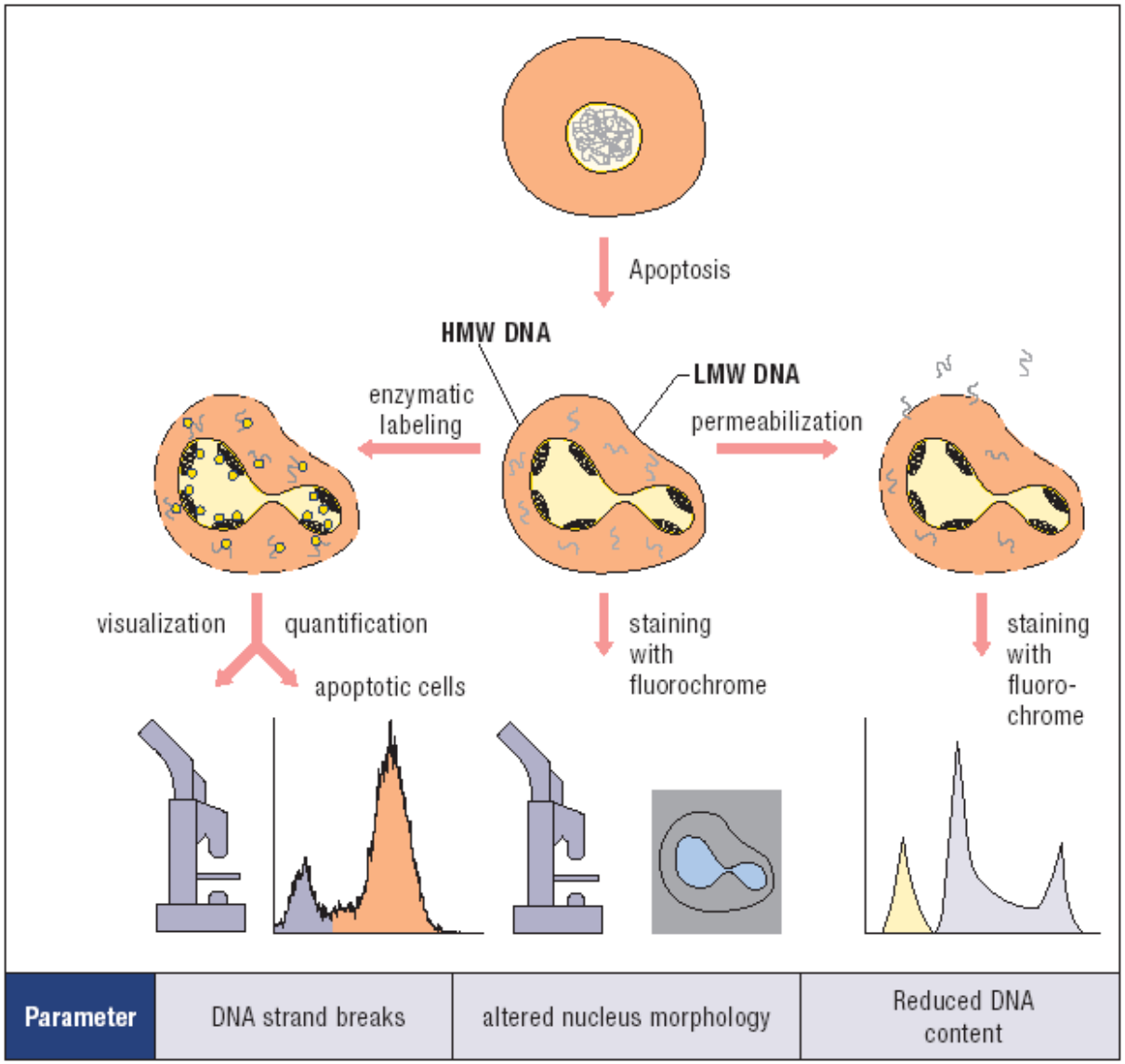
DNA labeling assay (using fluorescent probes assay)

Two types of nonviable cells

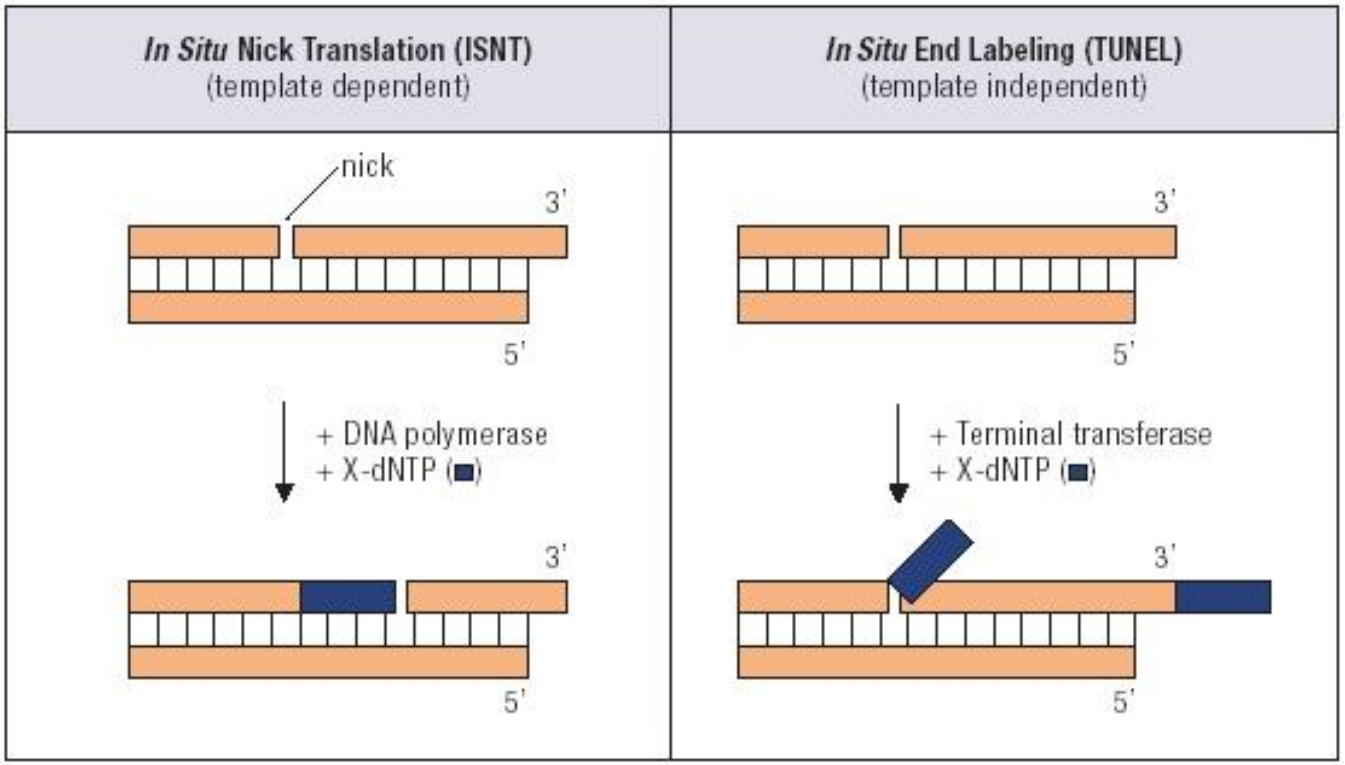


Two methods:

Enzymatic DNA labeling and DNA-binding dye labeling

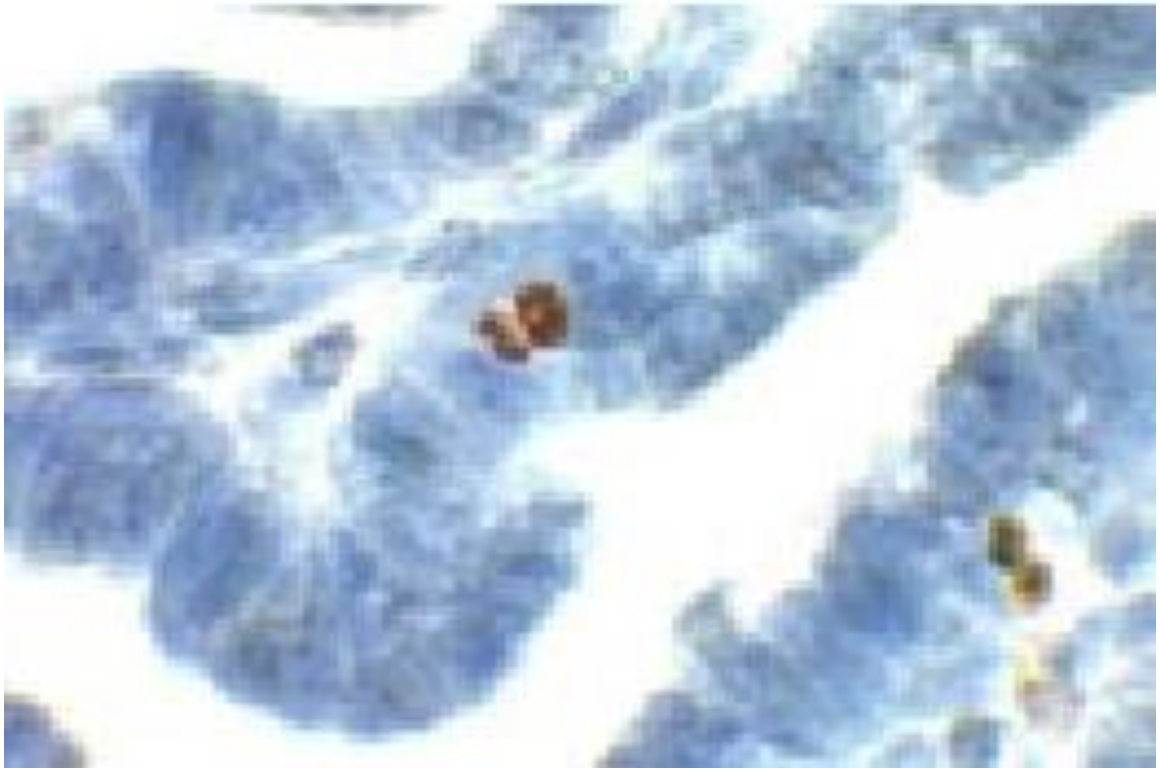


I. Enzymatic DNA labeling

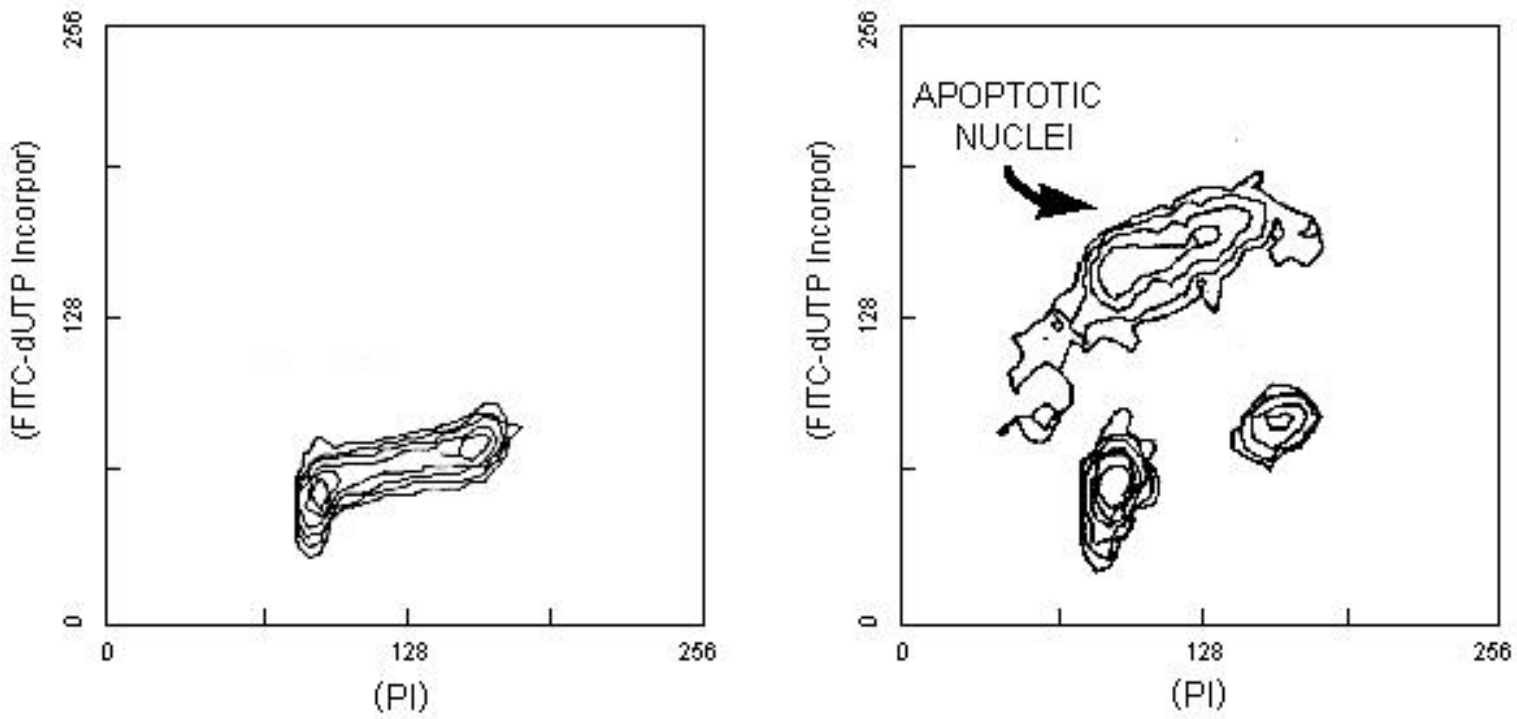


dNTP	dUTP		
	Direct	Indirect	
X	Fluoresein, FITC, PE etc	Biotin	DIG
		Avidin conjugated with fluoresein, avidin protein (AP), phycoerythrine, -pero xidase (POD),	**Anti-DIG antibody conjugated with fluoresein, AP, POD**

Anti-Digoxigenin antibody



Immunostaining of apoptotic cells (dark brown) by TUNEL and peroxidase staining in rabbit endometrium



Flow cytometric histograms of control (left) and apoptosis-induced cells (right) by PI and TUNEL labeling in HL-60 myeloid cells

Ⅱ. DNA-binding dye labeling

DNA-binding dyes: fluorochrome

Dye	Permeability via intact membrane	Staining	
		DNA	RNA
Acridine orange	Yes	Green	Red-orange[1]
Hoechst 33342	Yes	Blue	No
Hoechst 33258	Yes	Blue	No
DAPI (4,6-diamidino-2-phenylindole)	Yes	Bright blue	No
EtBr (Ethidium bromide)	No	Orange	Slightly red[1]
PI (Propidium iodide)	No	Red	No[2]

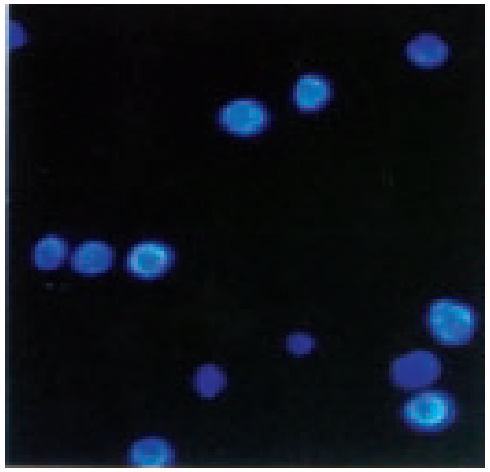
[1] RNase treatment is required

[2] RNase treatment is required because PI could stain double strand RNA

Pattern of dye staining according to color and chromatin morphology

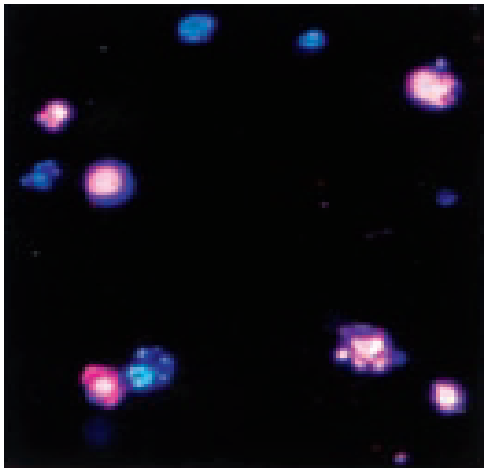
Dye	Apoptosis		Necrosis
	Early apoptosis	Late apoptosis*	
Acridine orange	Green Condensed	Green Fragmented	Green Diffuse, Intact
Hoechst 33342	Blue Condensed	Blue Fragmented	Blue Diffuse, Intact
Hoechst 33258	Blue Condensed	Blue Fragmented	Blue Diffuse, Intact
DAPI	Blue Condensed	Blue Fragmented	Blue Diffuse, Intact
Ethidium bromide	No (Orange, Condensed if permeabilized)	Orange Fragmented	Orange Diffuse, Intact
Propidium iodide	No (Red, Condensed if permeabilized)	Red Fragmented	Red Diffuse, Intact

* late apoptosis is regarded as the stage of membrane fragmentation and secondary necrosis



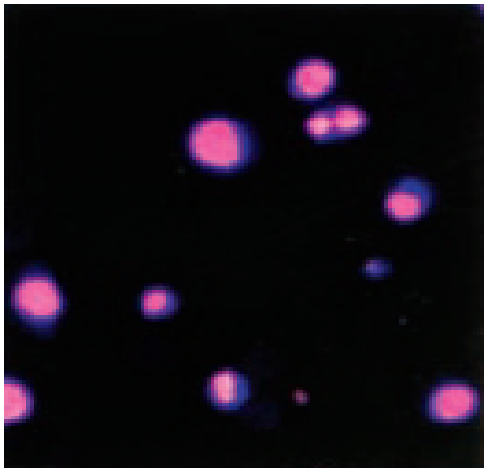
Apoptotic(0%)

Necrotic(10.5%)



Apoptotic(85.2%)

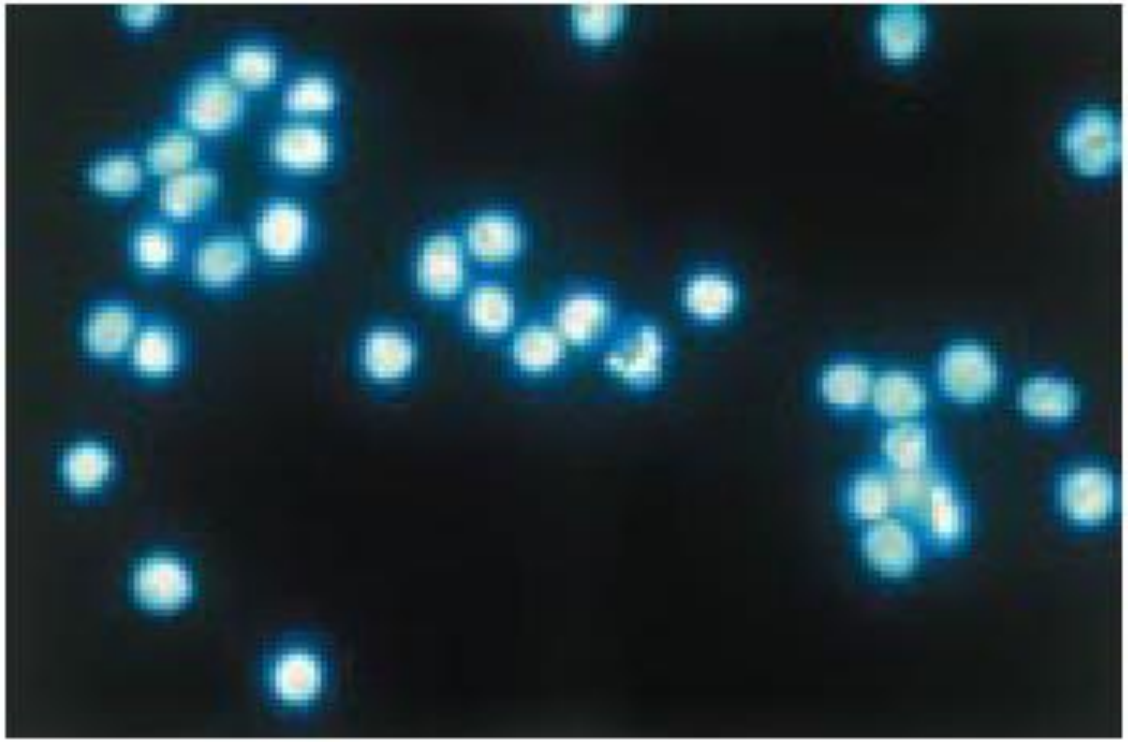
Necrotic(11.2%)



Apoptotic(1.2%)

Necrotic(92.5%)

In Hoechst 33258 / PI double staining, cells with blue intact nuclei were viable cells, whereas those with blue fragmented nuclei were early apoptotic cells. Cells with pink intact nuclei were necrotic cells, whereas cells with pink fragmented nuclei were late apoptotic cells. (blue against Hoechst33258, red against PI)



DAPI staining of condensed nuclei of apoptotic cells

- **Fluorescent protein biosensors measuring the molecular dynamics of macromolecules, metabolites, and ions in single cells have emerged from the integrative use of contemporary synthetic organic chemistry, biochemistry, and molecular biology**

- **Vascular endothelial cells (ECs) play and important role in physiologic hemostasis and blood vessel permeability, express immune related functions in monocytes and macrophages, and the viability of ECs is important in predicting the post-operative function and durability of cryopreserved vessels for implantation**

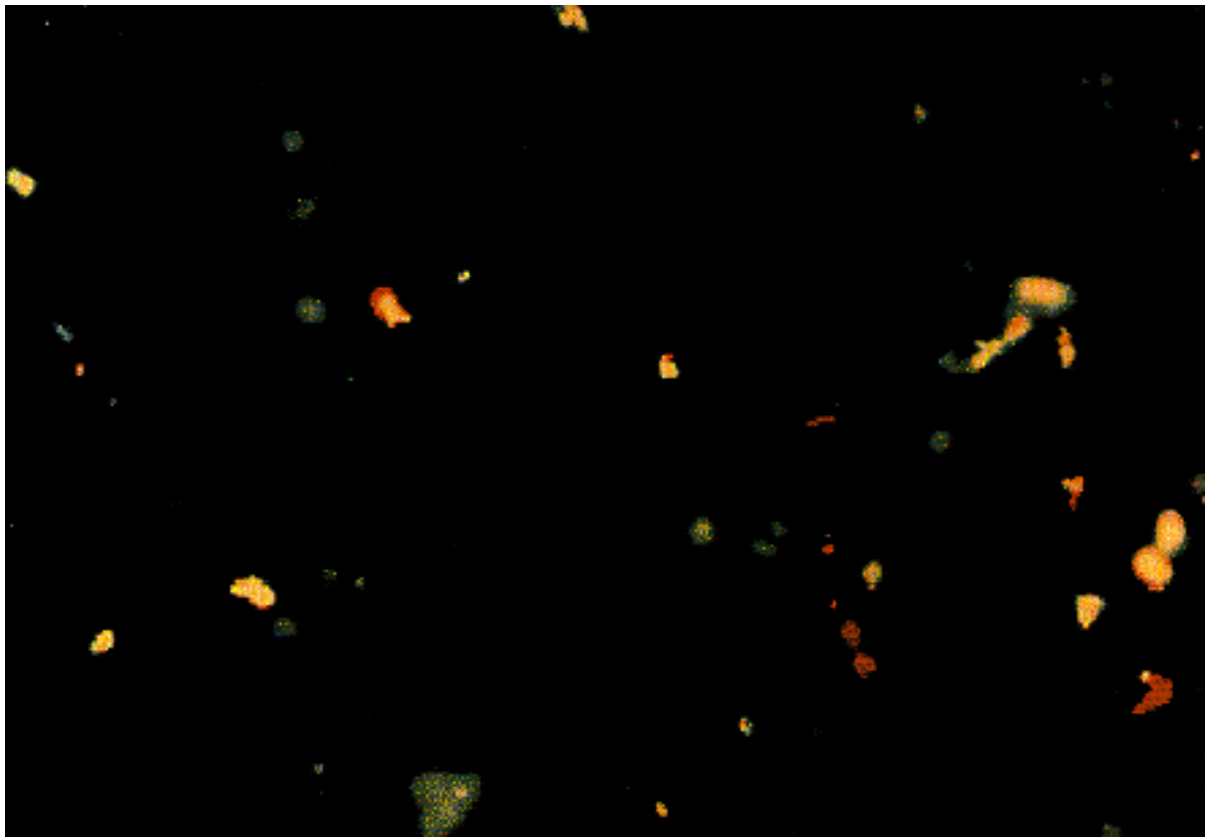
- **Tetrameric *Griffonia simplicifolia agglutins* (GS1) shows prominent binding only to the a-D-galactosyl residue of blood vessel ECs**

- **Staining with fluorescein isothiocyanate (FITC) conjugated with GS1 differentiates ECs from the other cells in flow cytometry**

- **PI intercalates DNA double strands in dead cells without regard to cell types, as their membrane lose integrity**

- **GS1-FITC and PI double staining: presumed to immediately determine the differential viability of ECs from whole cells**

- **The use of fluorescent probes enables the rapid determination of the viability of ECs and whole cells from the same tissue without separating ECs from whole cells, and of the viability of each step in the cryopreservation process**



Each part of quadrant statistics was observed under fluorescence microscopy. Live ECs preferentially expressed the green color of GS1. Dead ECs are double stained by the green color of GS1-FITC and the red color of PI, which results in yellow. Dead cells except dead ECs are identified by only the red color of PI

Morphological assay

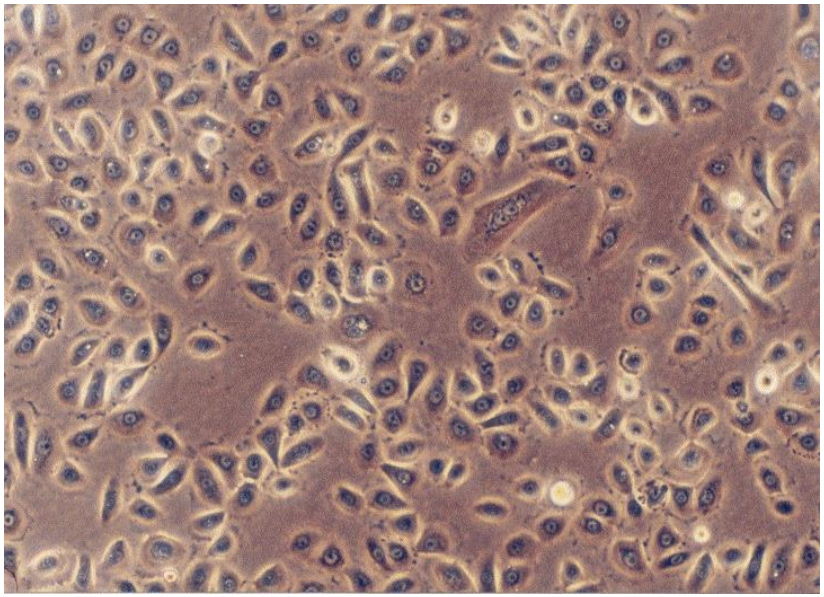
- Large-scale, morphological changes that occur at the cell surface, or in the cytoskeleton, can be followed and related to cell viability

- Damage can be identified by large decreases in volume secondary to losses in protein and intracellular ions of due to altered permeability to sodium or potassium

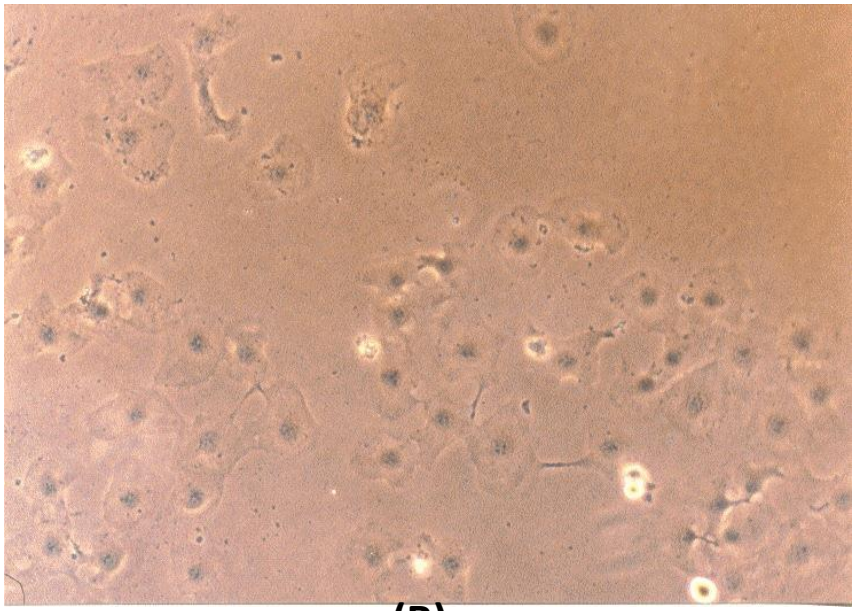
- Necrotic cells: nuclear swelling, chromatin flocculation, loss of nuclear basophilia

- Apoptotic cells: cell shrinkage, nuclear condansation, nuclear fragmentation

Example: Morphological feature (Human skin keratinocyte)



(A)



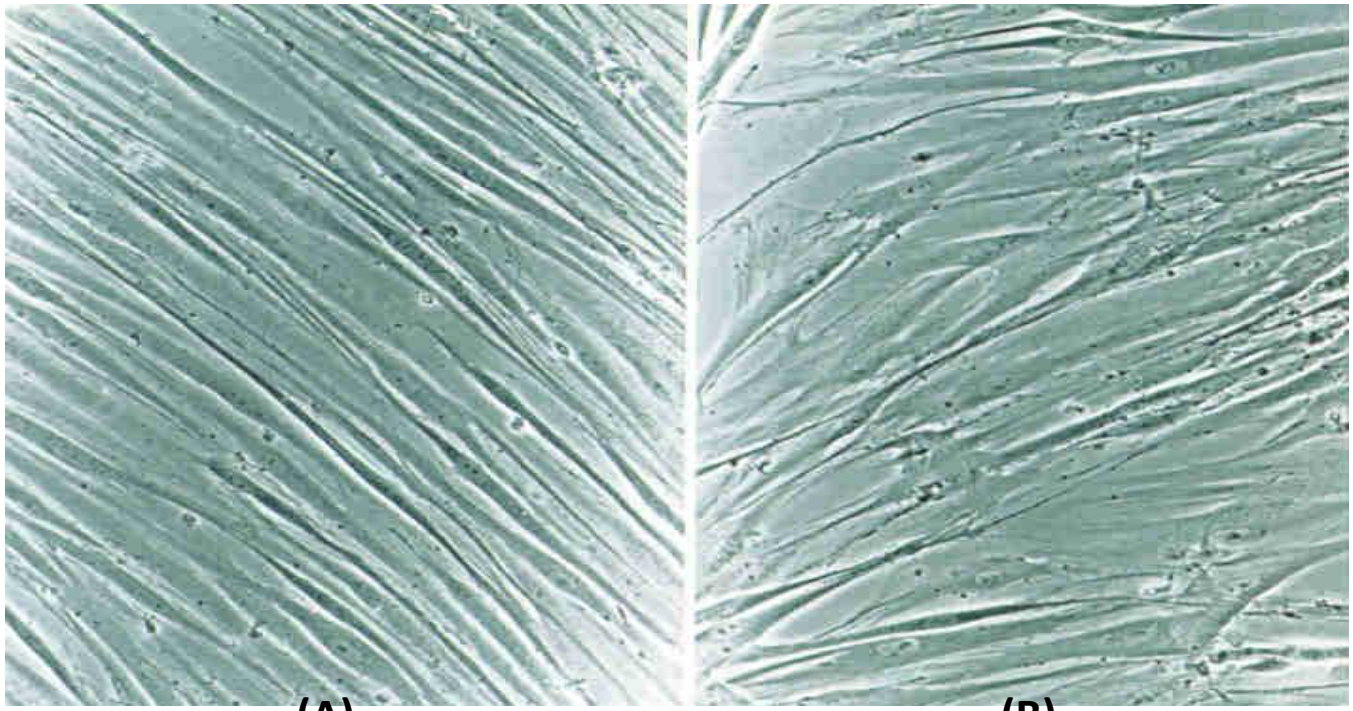
(B)

Morphological feature of

(A) normal human skin keratinocyte

(B) differentiated human skin keratinocyte

Example: Morphological feature (Human skin fibroblasts)



(A) (B)

Morphological feature of

(A)normal human skin fibroblasts,
(B)(B)aging human skin fibroblasts

Reproductive Assay

Colony-forming Efficiency

Clonogenic Cell:

- **Defined as a cell with the capacity for sustained proliferation**
- **Have undergone a minimum of 5-6 doublings to give rise to colonies containing at least 50 cells**

Colony-forming Efficiency (CFE)

- The ability to form colonies is used as a measure of reproductive integrity
- It is often referred to as plating efficiency (PE)

$$CFE = \frac{Number\ of\ colonies\ formed}{Number\ of\ cell\ plates} \times 100\%$$

Clonogenic assays

- Be used to reflect stem cell content
- The basis of assays for determining the lethal effects of cytotoxic agents

Determining the PE of an established adherent cell line

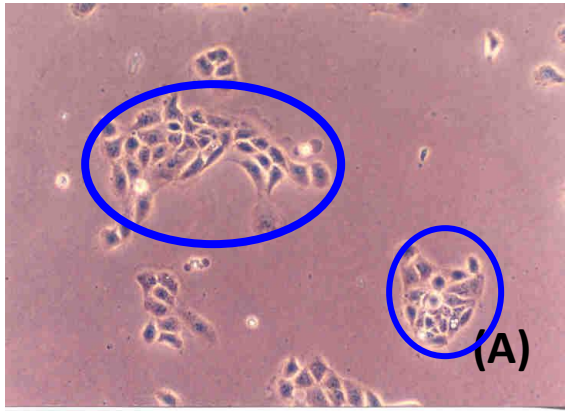
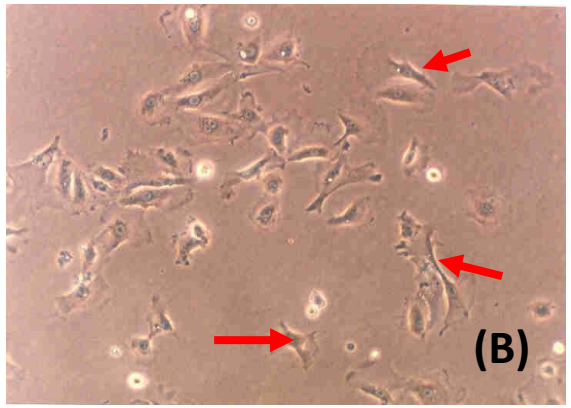
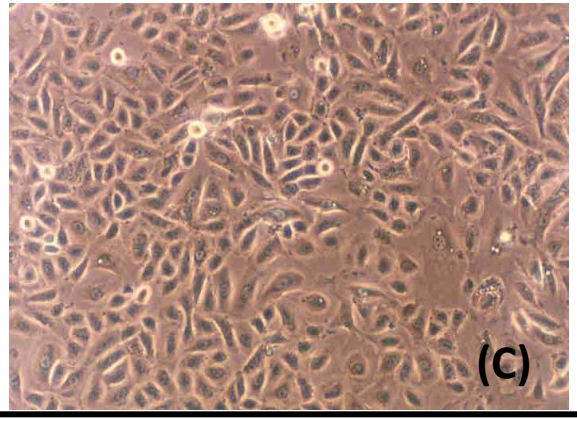
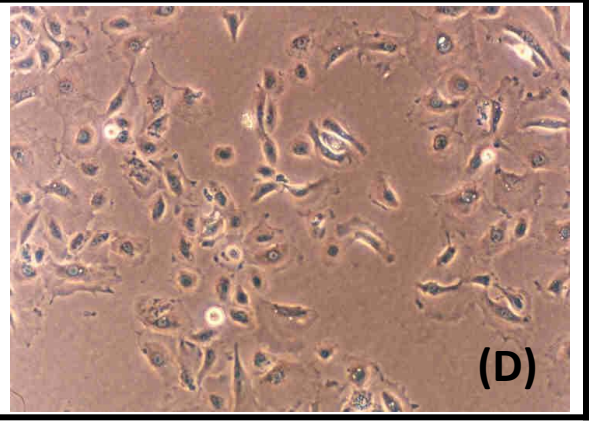
Materials and Equipment

- **Cell growth medium : Eagle's basal medium (BME)**
  100 iu/ml penicillin,
  0.1 mg/ml streptomycin
- **Trypsin-EDTA**
- **Gentain violet stain**

Procedure

1. Trypsinize monolayer cultures or use cell suspension cultures and determine the viable cell count
2. Dilute cells in growth medium to 1000 , 2000 and 5000 cells/10ml
3. Inoculate nine replicate Petri dished with 4 ml growth medium plus 1ml cell suspension
4. Place plates in a humidified 5% CO_2 plus air incubator are normal growth temperature and rock shelf or tray gently to and fro three times. The plates must not be moved now until colonies are stained
5. Stain and count three replicate per cell density at 1,2 and 3 weeks (murine lines) or 2 , 3 and 4 weeks (human lined)
6. Calculate the optimum cell densities for seeding and duration of incubation

Example: Rat keratinocytes

	Colony forming	*Non-colony forming*
48 hr after subculture		
6 days after subculture		

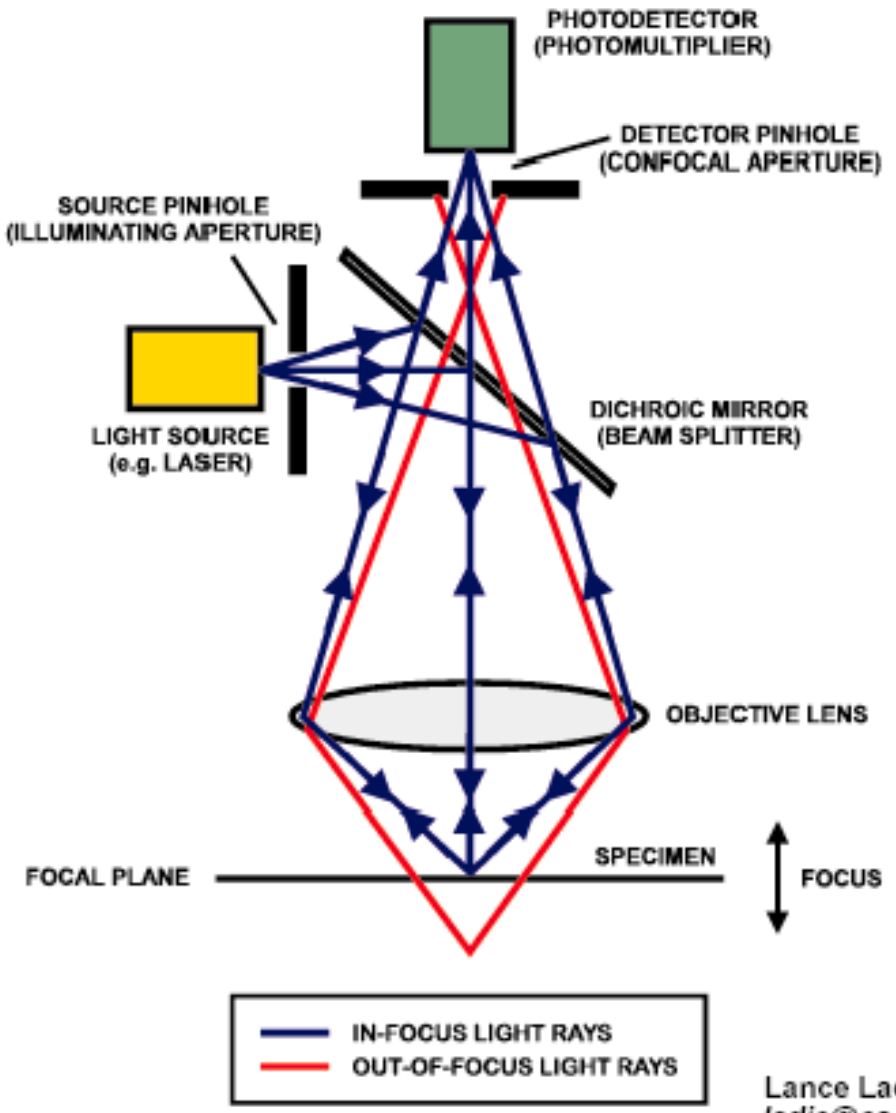
: colony , : Single cells

Laser Scanning Confocal Microscopy: 3-D tissue viability assay

Laser Scanning Confocal Microscopy

- Laser scanning confocal microscope (LSCM) is capable of observing selected thin layers of a thick specimen placed under a microscope. As a result, confocal images have significantly less fluorescence blur and out-of-focus light and resolution as well as contrast are improved
- This capability of selectively observing thin layers of a specimen is called 'optical sectioning'. A series of confocal sections can be combined into a three-dimensional image
- Combined use of LSCM and fluorescence provide powerful tools for intra-cellular phenomena and 3D tissue investigations

Simplified Optics of Laser Scanning Confocal Microscopy



Lance Ladic
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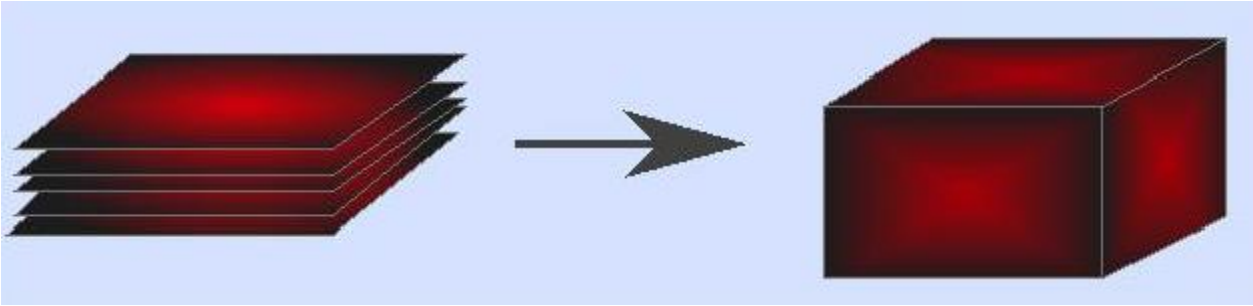
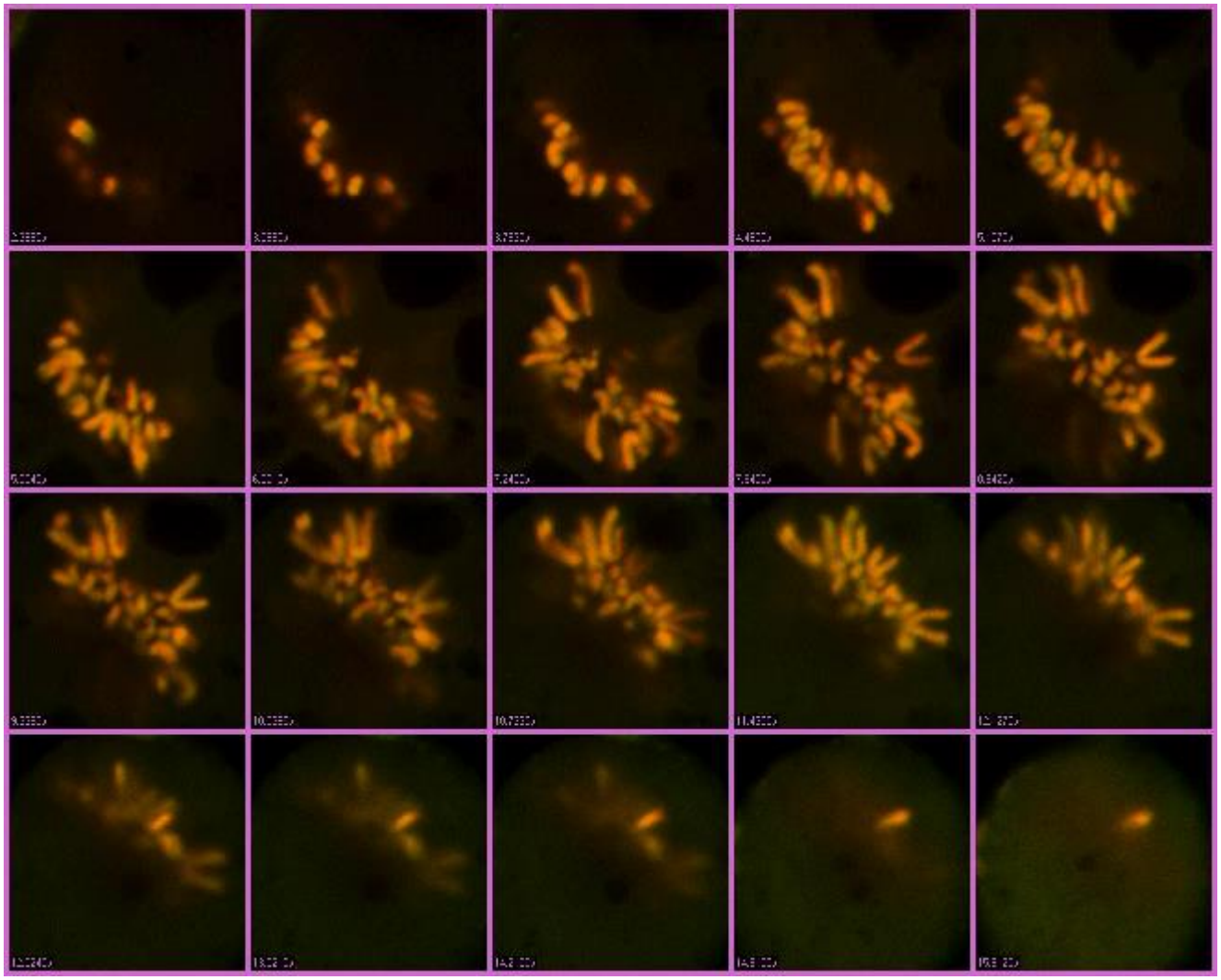
Principles of 3D image reconstruction



A series of confocal sections

A 3D image

Example of 3D image reconstruction

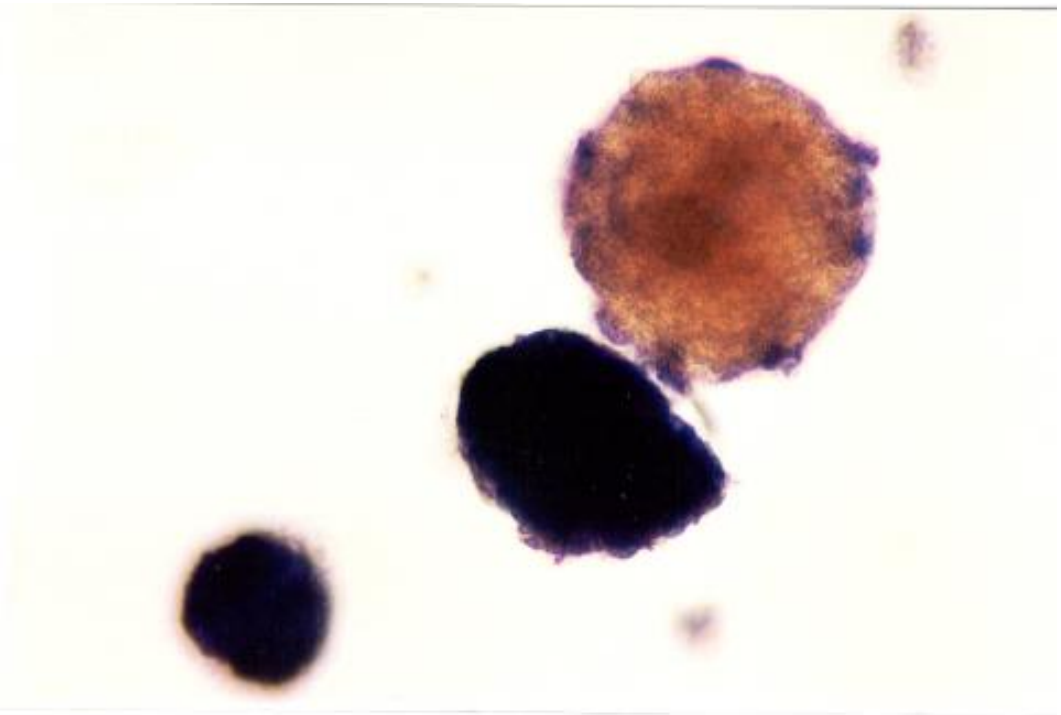


A series of confocal sections

A 3D image

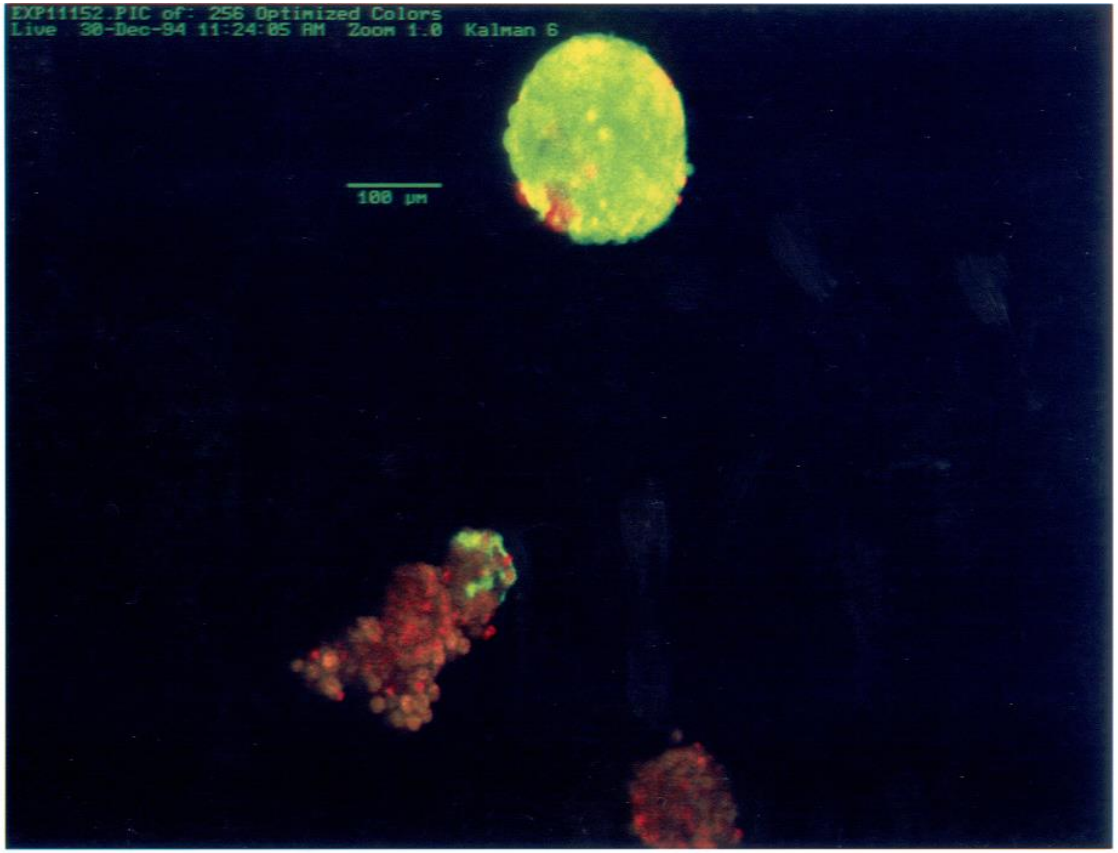
Example 2: 3-D viability of hepatoctye spheroids

- Rat primary hepatocyte spheorid 3-D viability: Qualitative (quantitative assay is also possible using 3-D image reconstruction)
- Olympus BH-2 microscope
- Epifluorescence linked with a MRC-500 confocal imaging system
- Illumination by argon ion laser
- Fluorescein diacetate(FDA)/Ethidium bromide(EB)
  - FDA: cleaved by esterase in cytoplasm of viable cell: fluoresces green
  - EB: inserted in DNA of dead cells ; fluoresces orange
  - FDA/EB signals: 510nm/560nm bandpass filter
  - Variable plane of optical sectioning: 10~15μm
  - Laser penetration dept: 50μm



Viability test of spheroid by trypan blue dye

Surface viability



Confocal microscopic picture of spheroid, day 15

3-D viability

Nuclear Magnetic Resonance (NMR) Methods

- Monitoring of cell metabolism
- 3-D observation of high density perfusion bioreactor

Introduction

- Nuclear magnetic resonance (NMR), which was discovered in 1946, was used primarily by organic chemists for elucidation of the structure of relatively small organic molecules

- NMR is now a proven technique for monitoring metabolism in diverse systems- isolated cells and perfused organs, as well as the intact animal and humans

- Studies of cell metabolism have generally utillized the ^{31}P, ^{13}C, ^{1}H, and ^{15}N nuclei

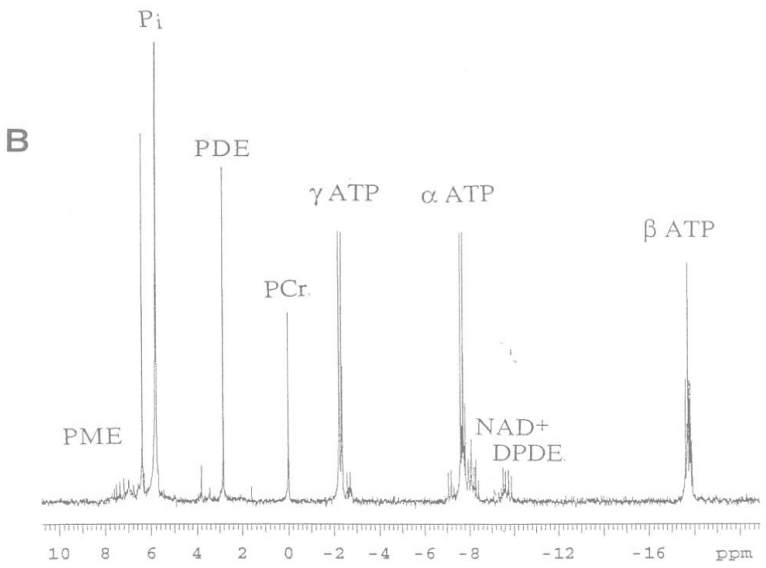
Applications

- **Spin-echo NMR image can be used to monitor fiber distribution in the hollow fiber bioreactor**
- **Diffusion-weighted images can be used to map cell distribution**
- **Flow imaging can be used to map flow rates in both the fibers and the extracapillary space.**
- **Chemical shift imaging can be used to map the distribution of cellular metabolites**
- **19F NMR imaging of a perfluorocarbon probe molecule can be used to map dissolved oxygen concentration**

Example ; Cellular energetics using ^{31}P NMR methods

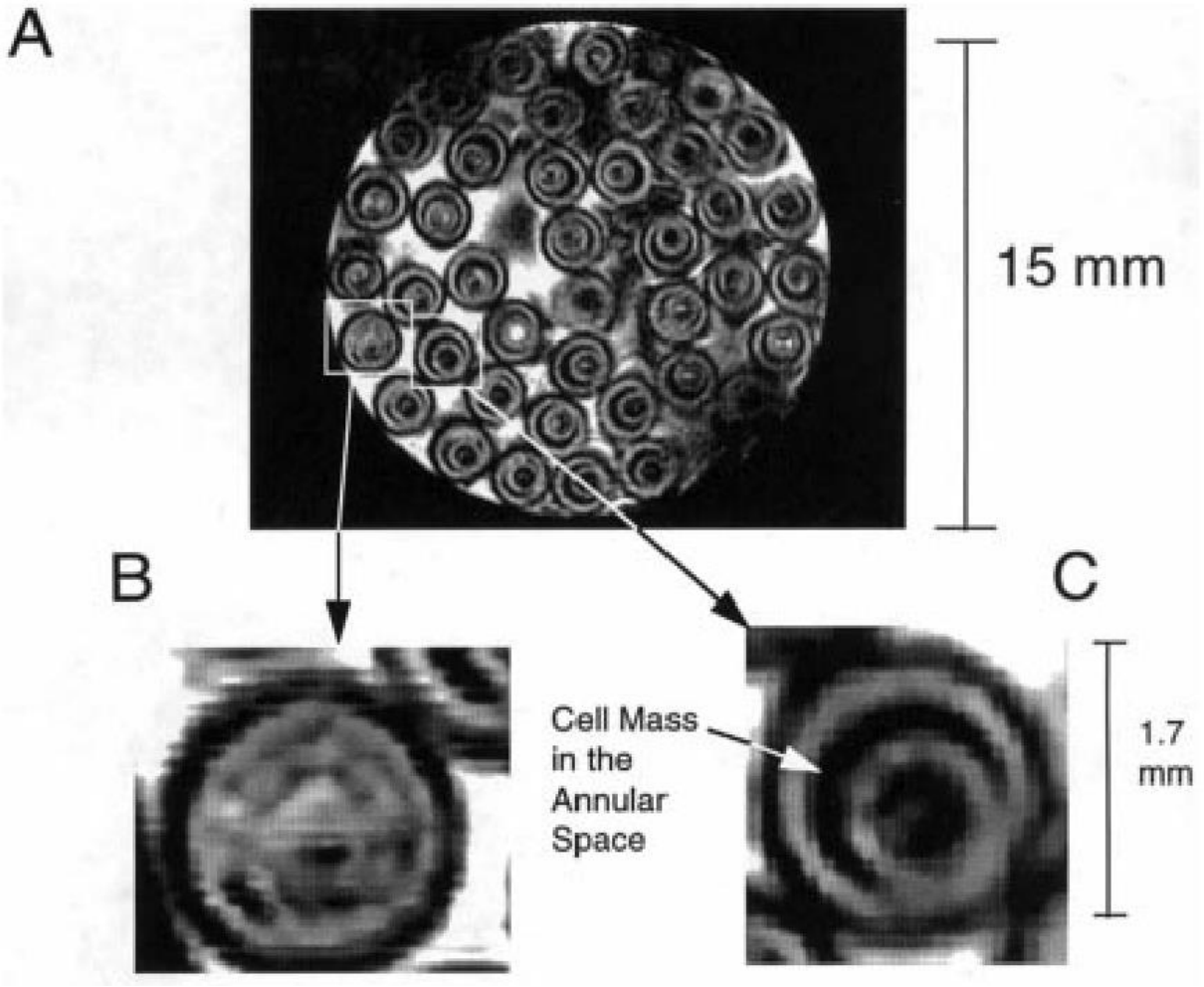
Procedure

1. Disslove cell extract in 5 mL of extract buffer and remove undissolved material by centrifugation.
2. Transfer 3.5 mL of the supernatant to a 10 mm-diameter NMR tube contatining 0.5 mL D_2O. In the case of ^{31}P NMR, MDP contained in a coazial tube can be used as a chemical shift and quantitation standard.
3. Acquire NMR spctrum, maintaining sample at a fixed temperature, typically 30℃. In the case of ^{31}P NMR, a 5 s interpulse delay and a 60° flip angle pulse is sufficient to ensure complete relaxation of the metabolite resonances.



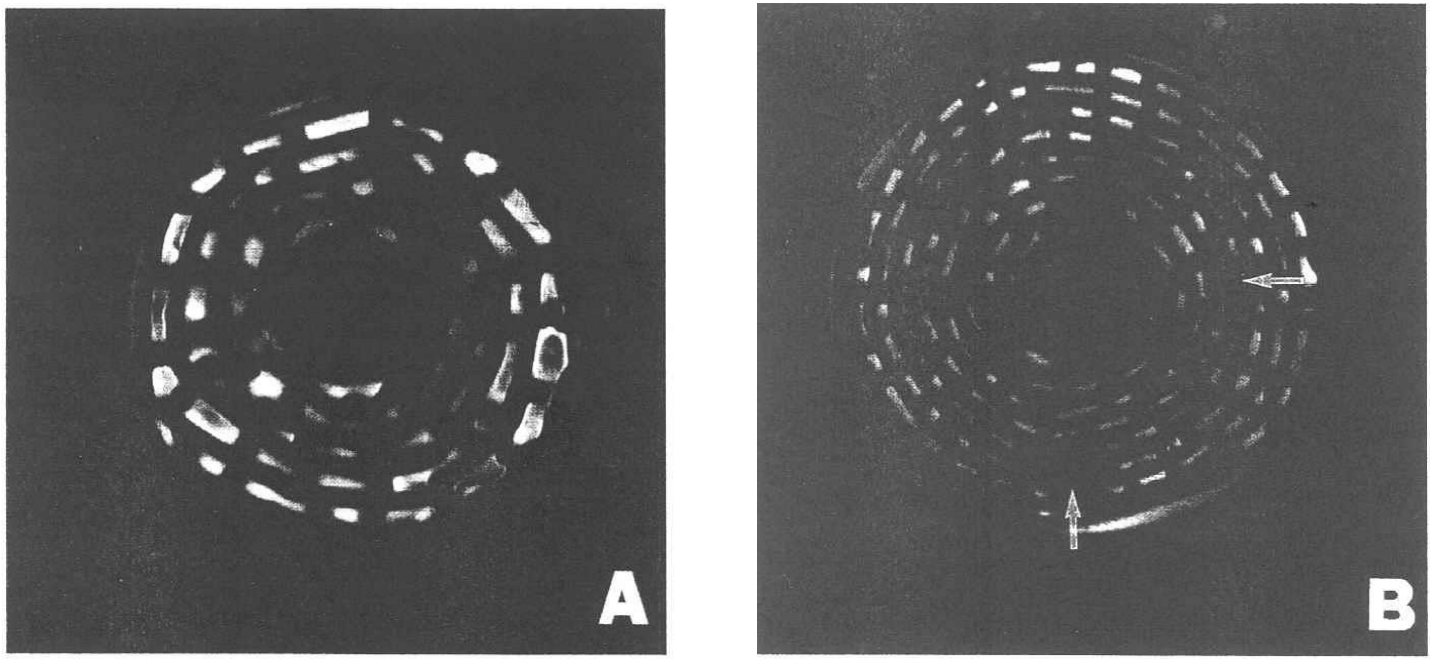
^{31}P NMR spectrum of a perchloric acid extract of CHO K1 cells growing in a hollow-fiber bioreactor. PME: phosphomonoesters, P_i: inorganic phosphate, PDE: phosphodiesters, PCr: phosphocreatine, ATP: adenosin triphosphate, NAD^+: nicotinamide adnine dinucleotide, DPDE: diphosphodiesters.

Example ; Cell distribution imaging



❖ **Transaxial T_2-weighted MRIs of coaxial hollow-fiber bioreactor for hepatocyte distribution examination. Nearly void (B) and full of hepatocytes (C).**

Example ; Fluid velocity distribution imaging



- **Transaxial flow sensitive MRIs of a small (A, intermal diameter 1.32 cm) and a scaled-up bioreator (B, internal diameter 2.2 cm). The fluid velocity ranged from zero (black) to around 2 cm/s (white).**

Cell toxicity

- Cytotoxicity causes inhibition of cell growth
- Observed effect on the morphological alteration in the cell layer or cell shape
- Characteristics of abnormal morphology is the giant cells, multinucleated cells, a granular bumpy appearance, vacuoles in the cytoplasm or nucleus
- Cytotoxicity is determined by substituting materials such as medium, serum, supplements flasks etc. at atime