FACULTY OF ENGINEERING & TECHNOLOGY

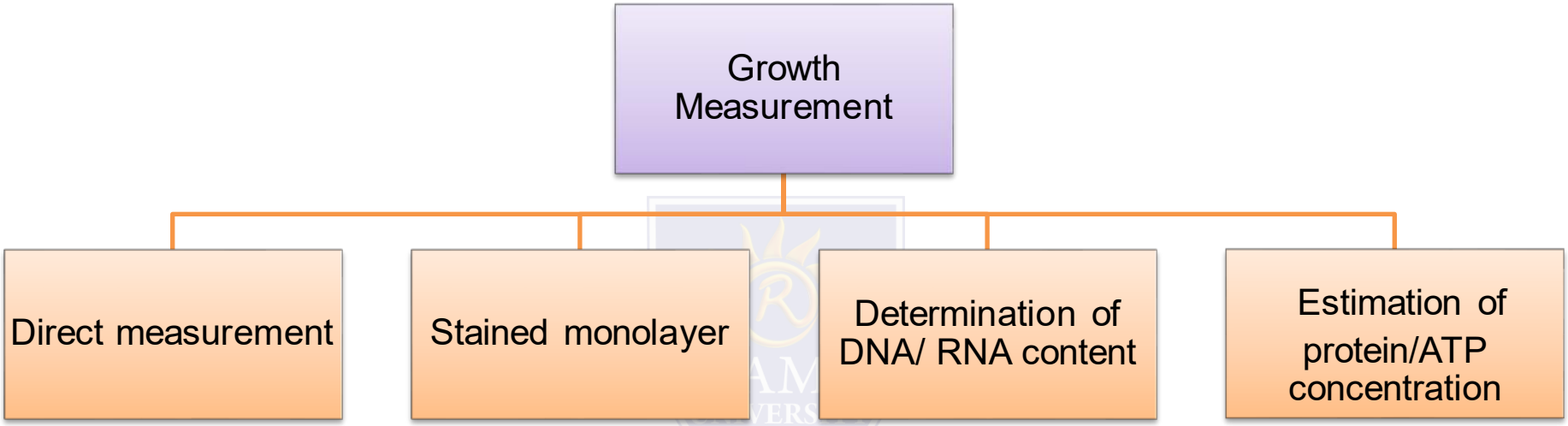
LT. 4. Measurement of Growth

Outline

1. Methods of growth Measurements
    a. Direct measurement
    b. Stained monolayer
    c. Estimation of DNA/ Protein content
    d. Estimation of protein/ ATP

LT. 4. Measurement of Growth

Measurement of growth is necessary to asses the health status of cultured cells. Further, it quantifies cellular growth and helps to establish reproducible culture condition.



Direct Measurement : Hemocytometer, Plating/plate count

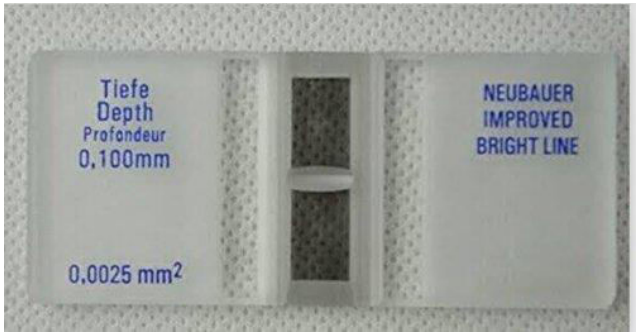
Determination of DNA/ RNA content : Fluorescent Dye DAPI, Hoechst stain

Estimation of protein/ATP concentration : Bradford assay, radioisotopically labeled amino acid, such as [3H]leucine or [35S]methionine followed by measurement of scintillation.

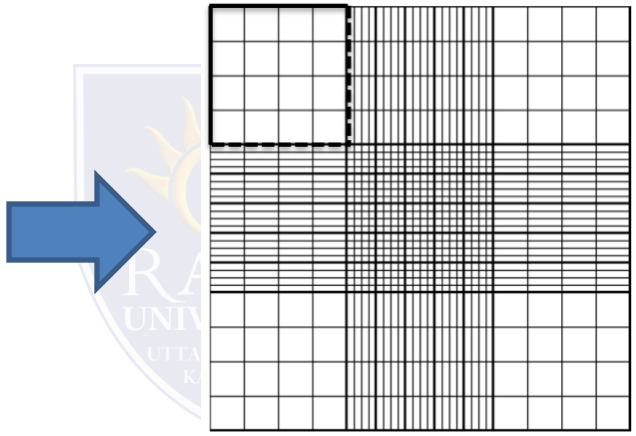
Hemocytometer

It is widely used measurement method of cell number and is coupled with dye exclusion assay using Trypan blue.

Hemocytometer is an especially fabricated glass slide which contains grid structure.



Typical Hemocytometer



Grided appearance under microscope (steel section on slide)

- Necessary volume for loading: 10-20 µl
- It is recommended that you count at least 100 cells
- Suspensions should be dilute enough so that the cells or other particles do not overlap each other on the grid, and should be uniformly distributed

$$\text{Total cells/ml} = \text{Total cells counted} \times \frac{\text{dilution factor}}{\text{\# of Squares}} \times 10{,}000 \text{ cells/ml}$$

Stained Monolayer

•This techniques is used when cells are too few to count in suspension.

•cells may be fixed and stained in situ and counted by eye with a microscope

•A rough estimate of the cell number per well can also be obtained by staining the cells with Crystal Violet and measuring the absorption on a densitometer.

•This method has also been used to calculate the number of cells per colony in clonal growth assays.

•For estimation of cell number calibration curve is needed with respect to cell number and absorbance value.

Determination of DNA/ RNA content

•DNA may be assayed by several fluorescence methods, including reaction with DAPI PicoGreen (assay kit from Molecular Probes), or Hoechst 33258.

•The fluorescence emission of Hoechst 33258 at 458 nm.

•It is increased by interaction of the dye with DNA at pH 7.4 and in high salt to dissociate the chromatin protein.

•This method gives a sensitivity of 10 ng/mL but requires intact double-stranded DNA.

Determination of protein content

•The protein content of cells is widely used for estimating total cellular material and can be used in growth experiments or as a denominator in expressions of the specific activity of enzymes, the receptor content, or intracellular metabolite concentrations.

•The amount of protein in solubilized cells can be estimated directly by measuring the absorbance at 280 nm, with minimal interference from nucleic acids and other constituents

Bradford assay

•The *Bradford* assay is based on the use of the dye Coomassie Brilliant Blue G-250, which is frequently abbreviated as Coomassie G-250 or Coomassie Blue.

•Coomassie Blue undergoes a spectral change on binding to protein in acidic solution

•The colour of Bradford solution changes from brown/red to blue on binding with protein molecules in sample.

•Color is generated in one step after a short incubation and should be read within 30 min using spectrophotometer.

•when the dye is red, Bradford reagent has an absorbance maximum (A_{max}) of 470 nm. In the presence of protein, the change to the anionic blue form of the dye shifts the A_{max} to 595 nm.

•The quantity of protein in a sample can measured directly by measuring the absorption at 595 nm

•Higher the protein, more will be the blue anionic form and higher will be the absorbance.

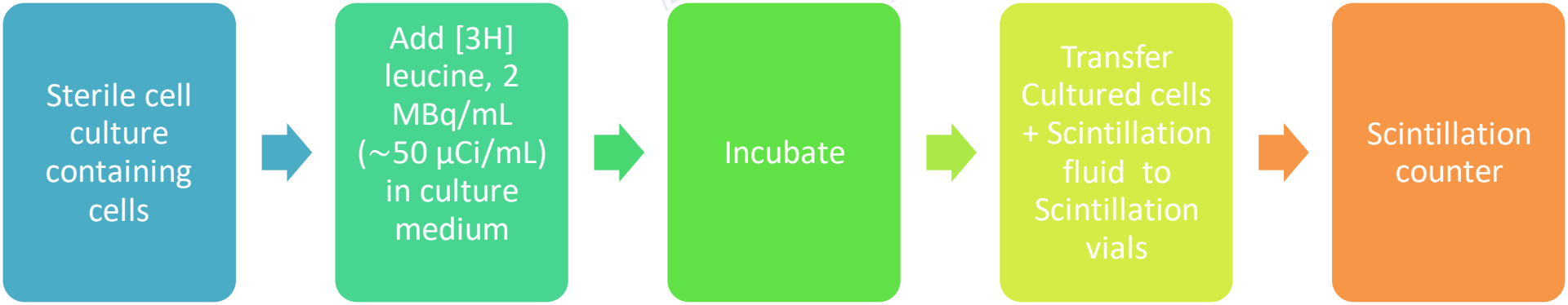
Determination of protein content

Radioisotopic method

Observations over a period of time may be used to measure the net protein accumulation or loss (i.e., protein synthesized–protein degraded).

Radioisotopically labeled amino acid, such as [3H] leucine or [35S] methionine, and measuring (e.g., by scintillation counting) the amount of radioactivity incorporated into acid-insoluble material per 1×10^6 cells or per milligram of protein over a set period of time.

Protocol steps to be followed



References & Further reading

1. https://vlab.amrita.edu/?sub=3&brch=188&sim=336&cnt=1
2. https://www.bio-rad.com/featured/en/bradford-assay.html
3. https://www.hemocytometer.org/hemocytometer-protocol/