EXERCISE - 6

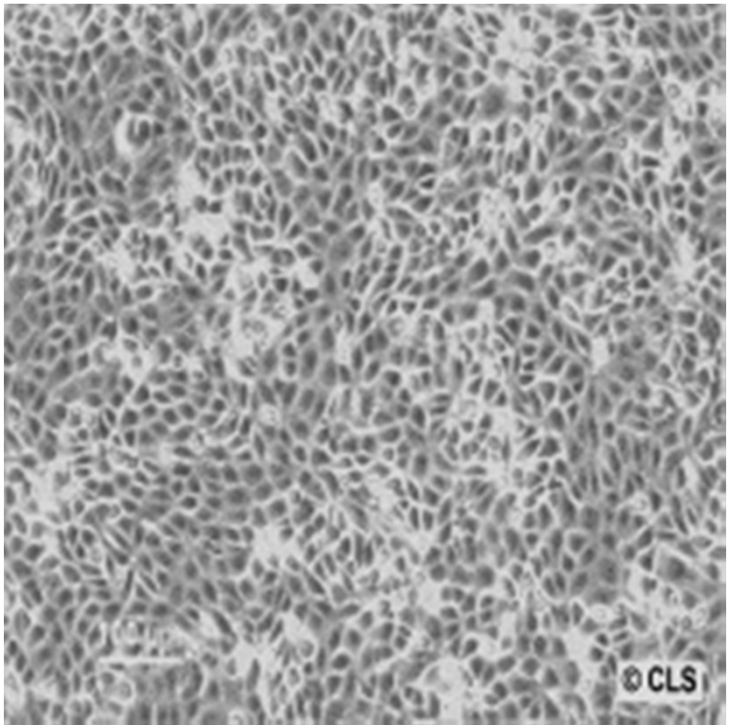
- **Counting of total cells as well as viable cells in MDBK cell suspension**

REQUIREMENT –

- Cell / tissue culture flask
- EMEM (Eagle's Minimum essential medium)
- FCS (Fetal calf serum)
- Growth medium (MEM with 5% FCS)
- Cell culture flask
- Haemocytometer (Neubauer's cell counting chamber)
- Cover slips
- Pipettes
- Eppendorf tubes (Micro tubes)
- Micro tips
- Inverted light microscope
- Upright light microscope
- Trypan blue dye
- Trypsin- versene solution
- Serum


canvax
Ref CC007
Lot D09161
Trypan Blue solution
0.4 % liquid, sterile-filtered,
suitable for cell culture
100 mL
MW 960.81 g/mol
Store at 25°C


1.5
1.0
0.5
1.5
1.0
0.5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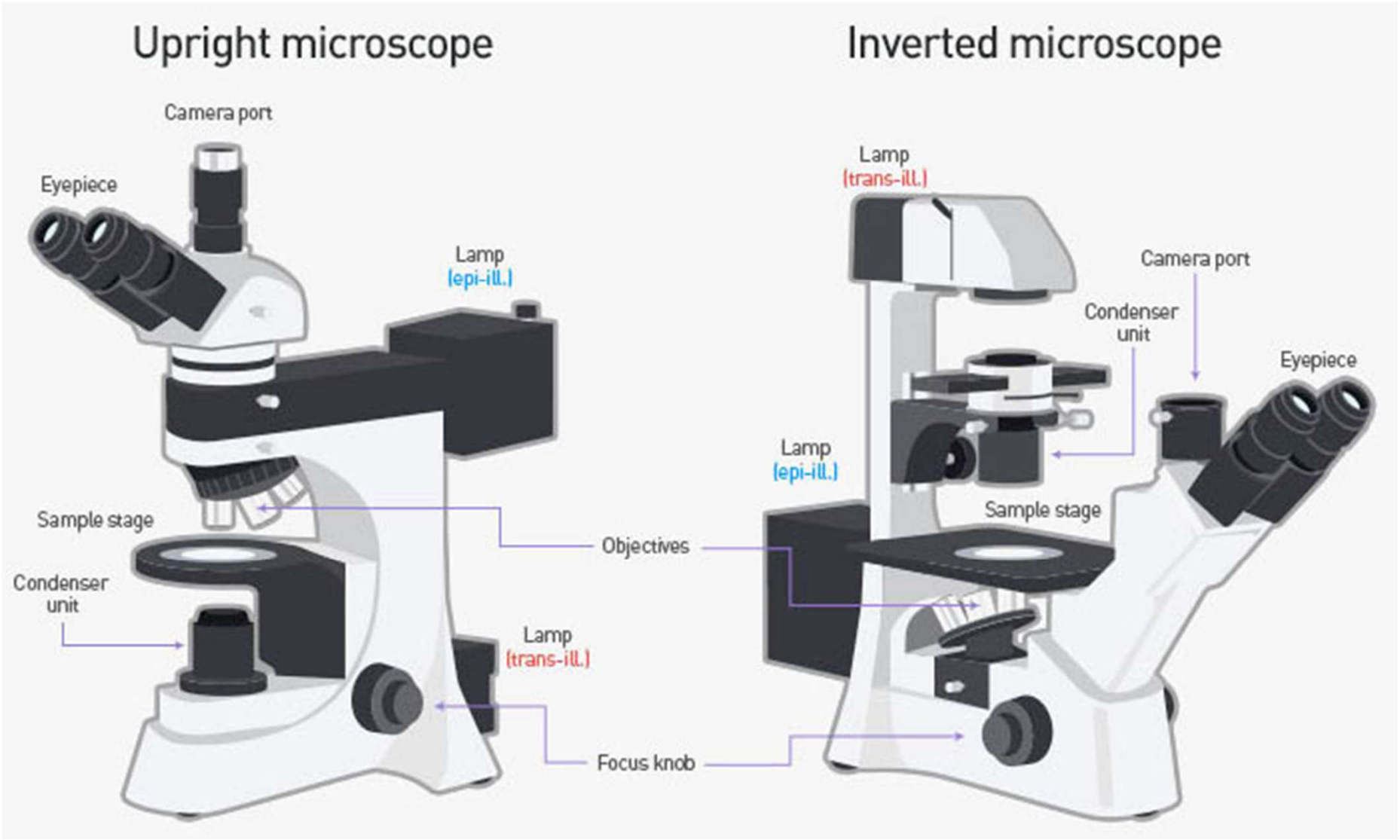
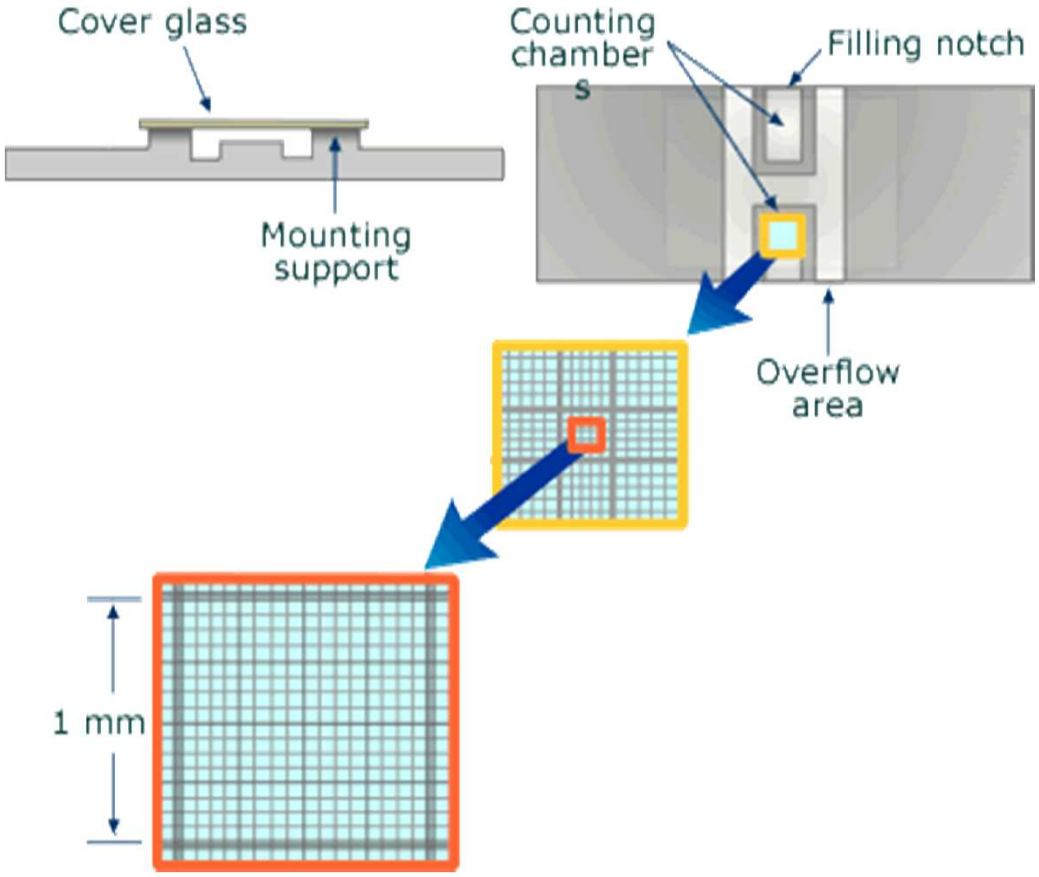
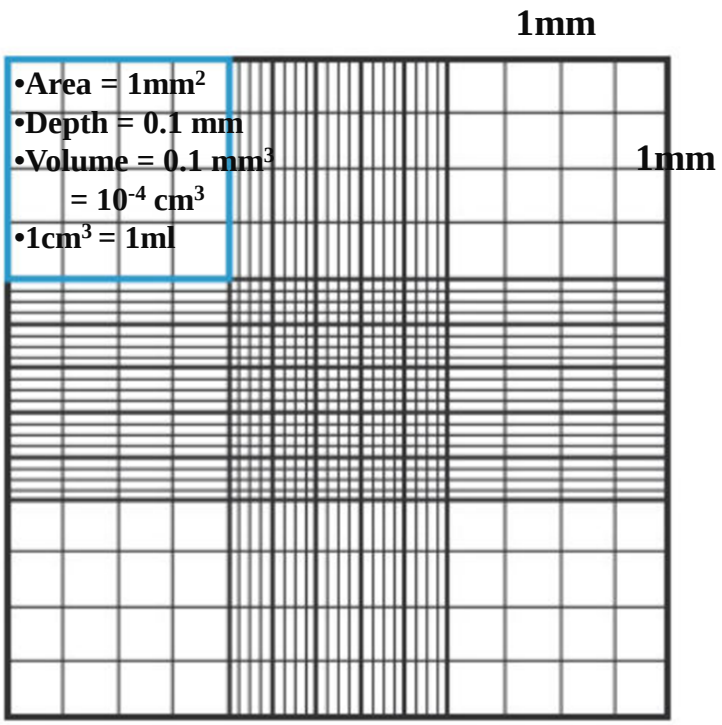

Upright microscope
Camera port
Eyepiece
Lamp
(epi-ill.)
Sample stage
Condenser
unit
Lamp
(trans-ill.)
Objectives
Focus knob
Inverted microscope
Lamp
(trans-ill.)
Camera port
Condenser
unit
Eyepiece
Lamp
(epi-ill.)
Sample stage

PROTOCOL –

1. Clean the laminar hood with cotton / tissue paper soaked with 70% ethanol and sterilize the hood for about 30 minutes by putting on the UV light.
2. Observe the cells in tissue culture flask sub-cultured 24 hrs before under the inverted microscope. (The cells should be healthy not over-confluent but in log / exponential phase).
3. Decant the medium and wash two times each with 1ml trypsin-versene solution prewarmed at 37°C.
4. Keep the flask at 37°C for 1-2 minute(s) until cells start detach from the surface.
5. Add 1 ml of growth medium and pipet the cells several times to make single cell suspension.
6. Take 0.1ml of cell suspension in an eppendorf tube and add 0.1ml of trypan blue (dilution = 10^{-2}).
7. Mix the cell suspension with the dye with micropipette tips.
8. Clean the haemocytometer and place a coverslip over the squares.
9. Charge the cell suspension with the help of pipette tips under the coverslip.

12. Use low power objective on microscope to count all the cells for total count (stained & unstained cells) and unstained cells for viable count.
13. Count the cells touching the right and bottom lines but not the top and left line.





•Area = 1mm²
•Depth = 0.1 mm
•Volume = 0.1 mm³
= 10^{-4} cm³
•1cm³= 1ml

CALCULATION - The number of viable cells in the original suspension is determined as follows :

- Suppose total cells and unstained cells (viable cells) in 5 squares (one central and 4 corners) are 195 and 165 respectively, then

Number of viable cells = Total number of unstained cells in 1 square (average count)

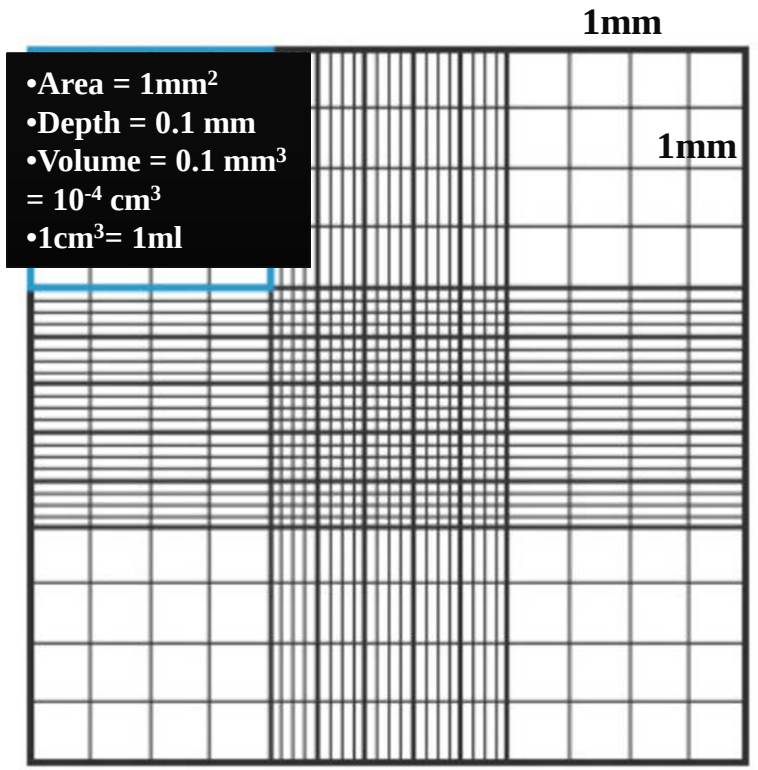
x Reciprocal of dilution factor / 10^{-4} cm^3

= 165/5 x 2 cells per 10^{-4} cm^3

= 66 cells per 10^{-4} ml

= 6.6 x 10^5 per ml

Number of total cells = 7.8 x 10^5 per ml



Dilution factor = (0.1 + 0.1) = 10^{-2}

Dilution factor = - 2

Reciprocal of dilution factor = 2