

- This is a most used haemocytometer is the one with the improved Neubauer counting chamber.
- Even though haemocytometer can be used to count most types of cells, but it was originally designed for counting blood cells.

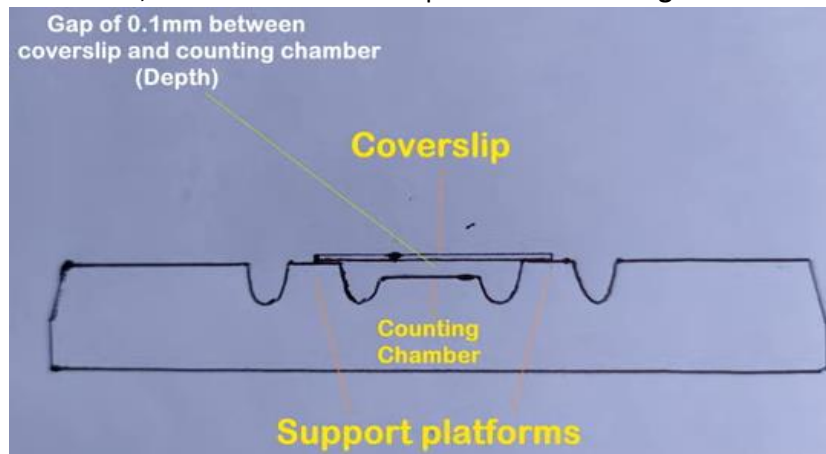
Specifications:

Complete set in a plastic case consisting of:

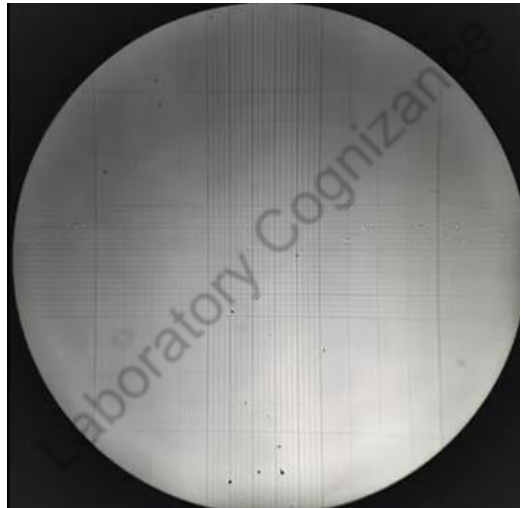
- One counting chamber equipped with a double counting grid.
- Two haemocytometer cover glasses with a thickness of 0.4 mm each.
- One blood diluting pipette, specifically the Thoma type, in both red & white colors.
- Two silicone tubings, each approximately 16 cm in length.
- One plastic adapter, available in both red & white variants.



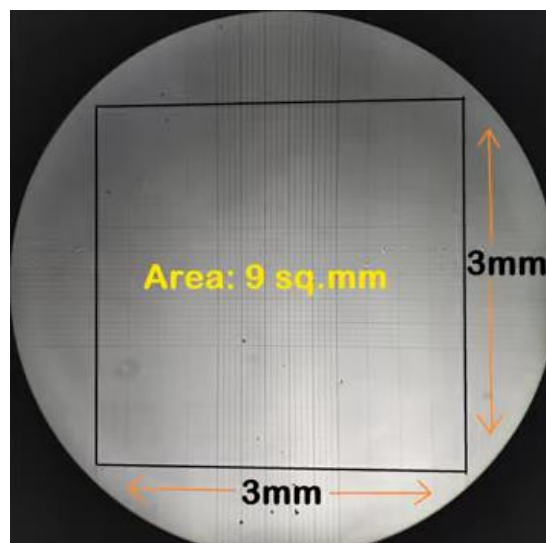
- The height of the platforms & the chambers are designed in such a way that when a coverslip is placed on the top there is a gap of 0.1 mm (millimeters) b/w the coverslip & the chambers, this is known as the depth of the “**Counting Chambers**”.



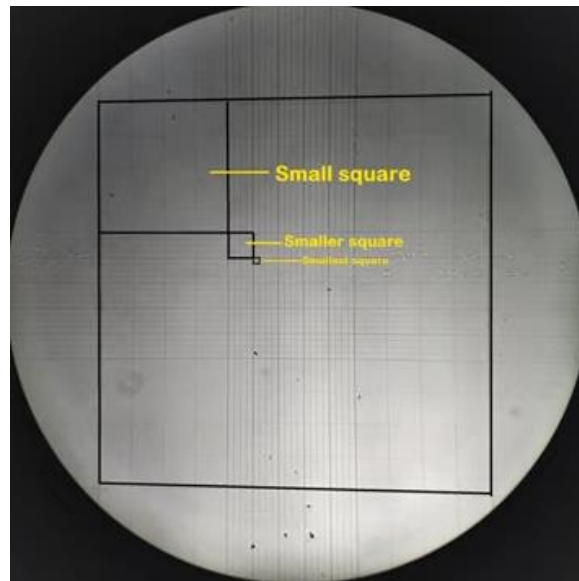
- This is how grid appears under the microscopic view:



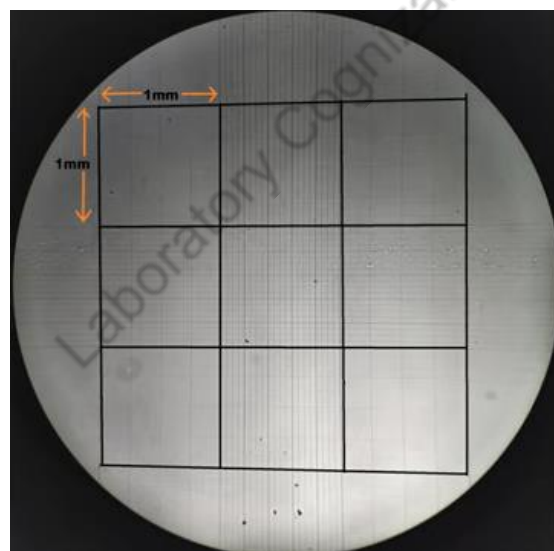
- The counting grid has overall dimension of 3 x 3 mm, with an area of 9 sq.mm.



- Within this counting grid can be found squares of three different sizes simply referred to as **small square**, **smaller square** & **smallest square**. (Remember)



- Now, the counting grid is consisting of 9 small squares, each with the dimension of 1 x 1 mm.



- Each of the four corner squares are further subdivided into 16 smaller squares, each measuring 0.25 x 0.25 mm & an area of 0.0625 square millimetres.

- **Mathematical details for upper values that is calculated:**

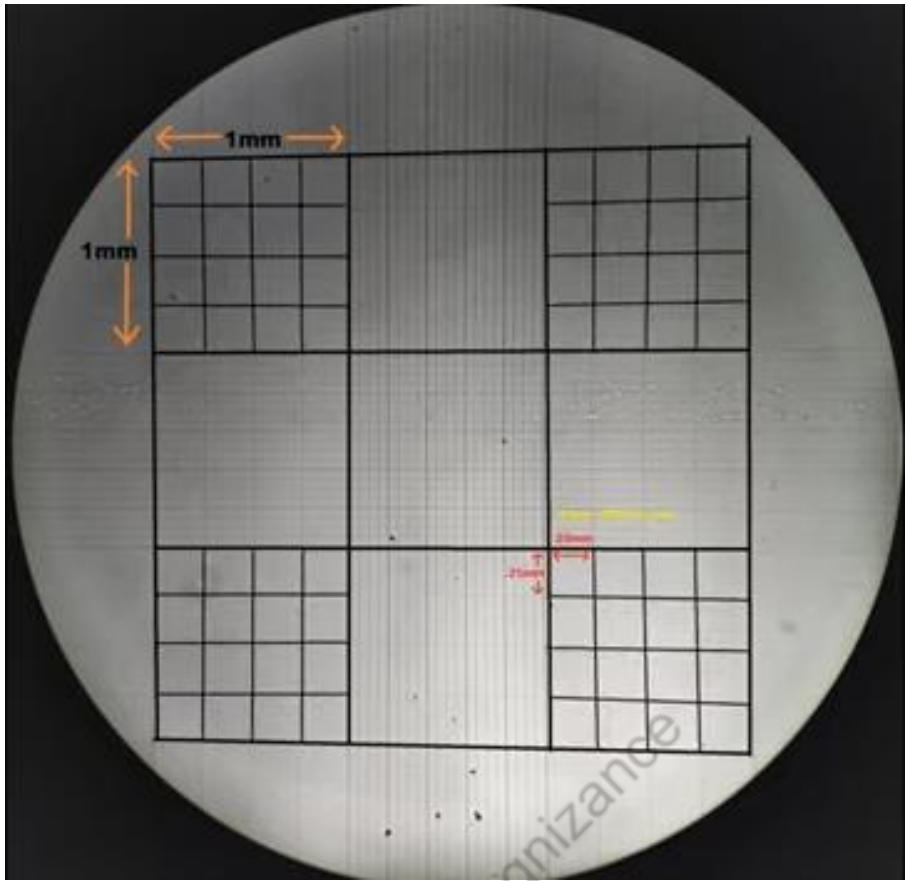
It is like this: $1 \text{ mm} / 4 = 0.25 \text{ mm}$ is width.

Now to measure the area of a single small square which is one of the 16 squares:

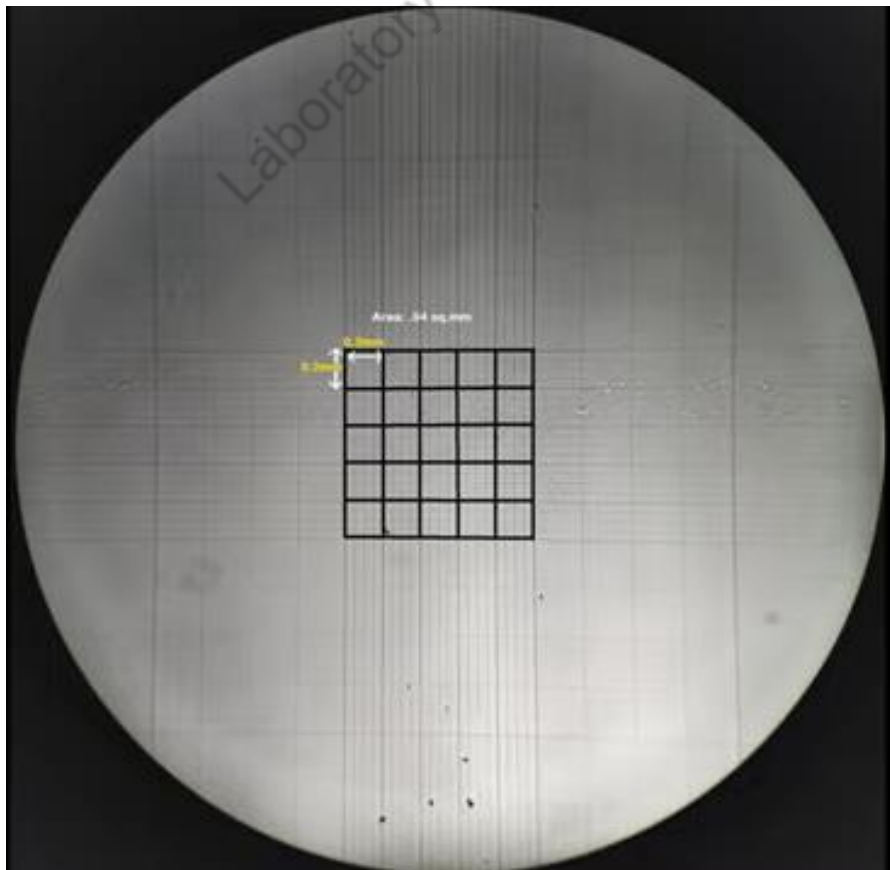
$0.25 \text{ mm} \times 0.25 \text{ mm} = 0.0625 \text{ square millimetres}$.

OR

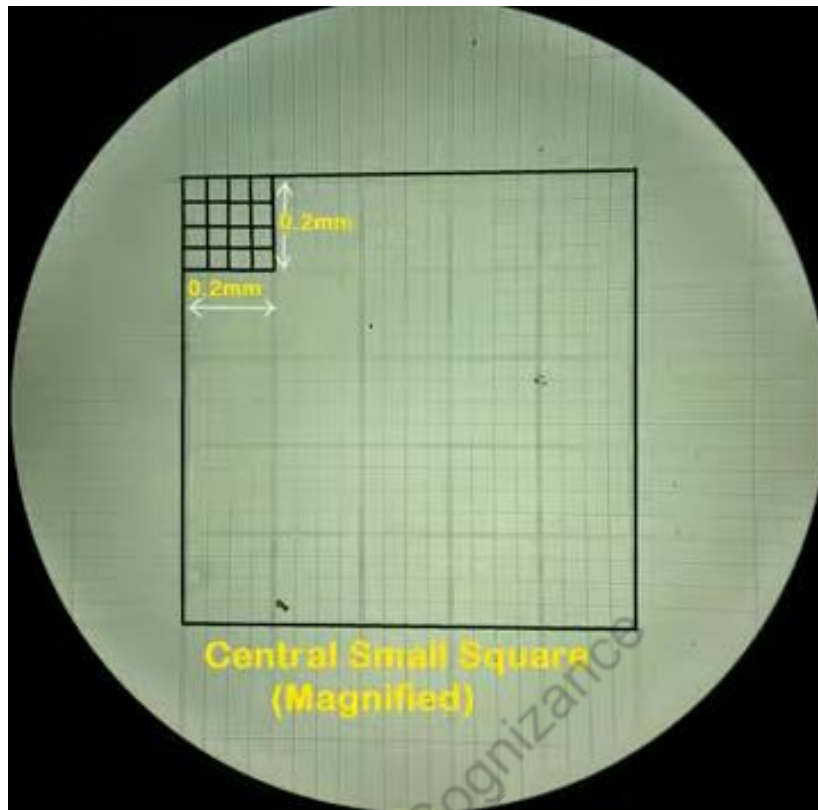
$1 \text{ mm}^2 / 16 = 0.0625 \text{ mm}^2$.



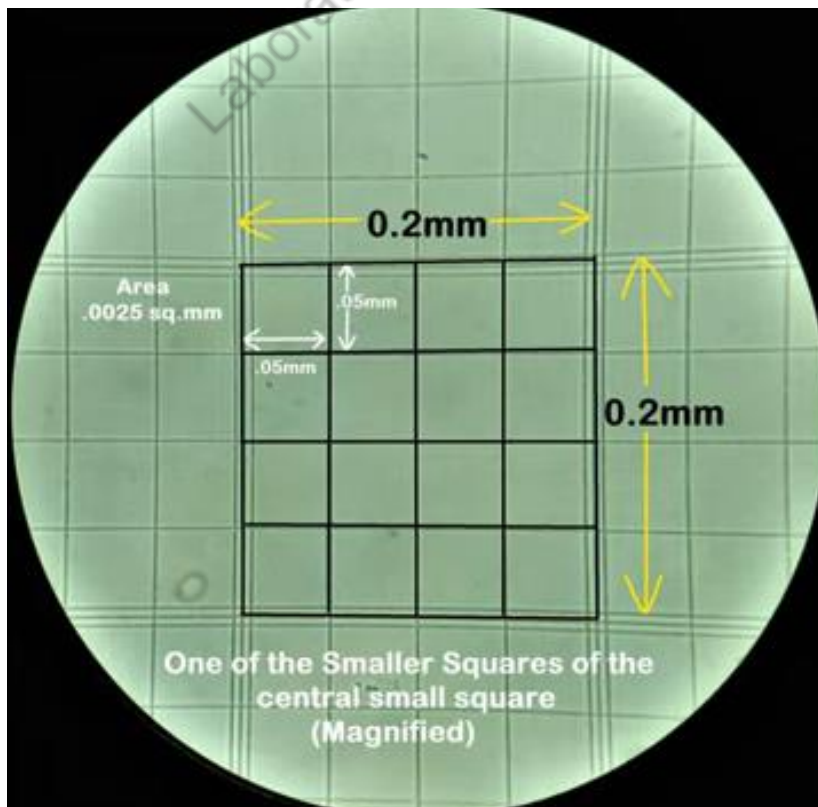
- The small square at the center of the grid is however divided into 25 smaller squares.



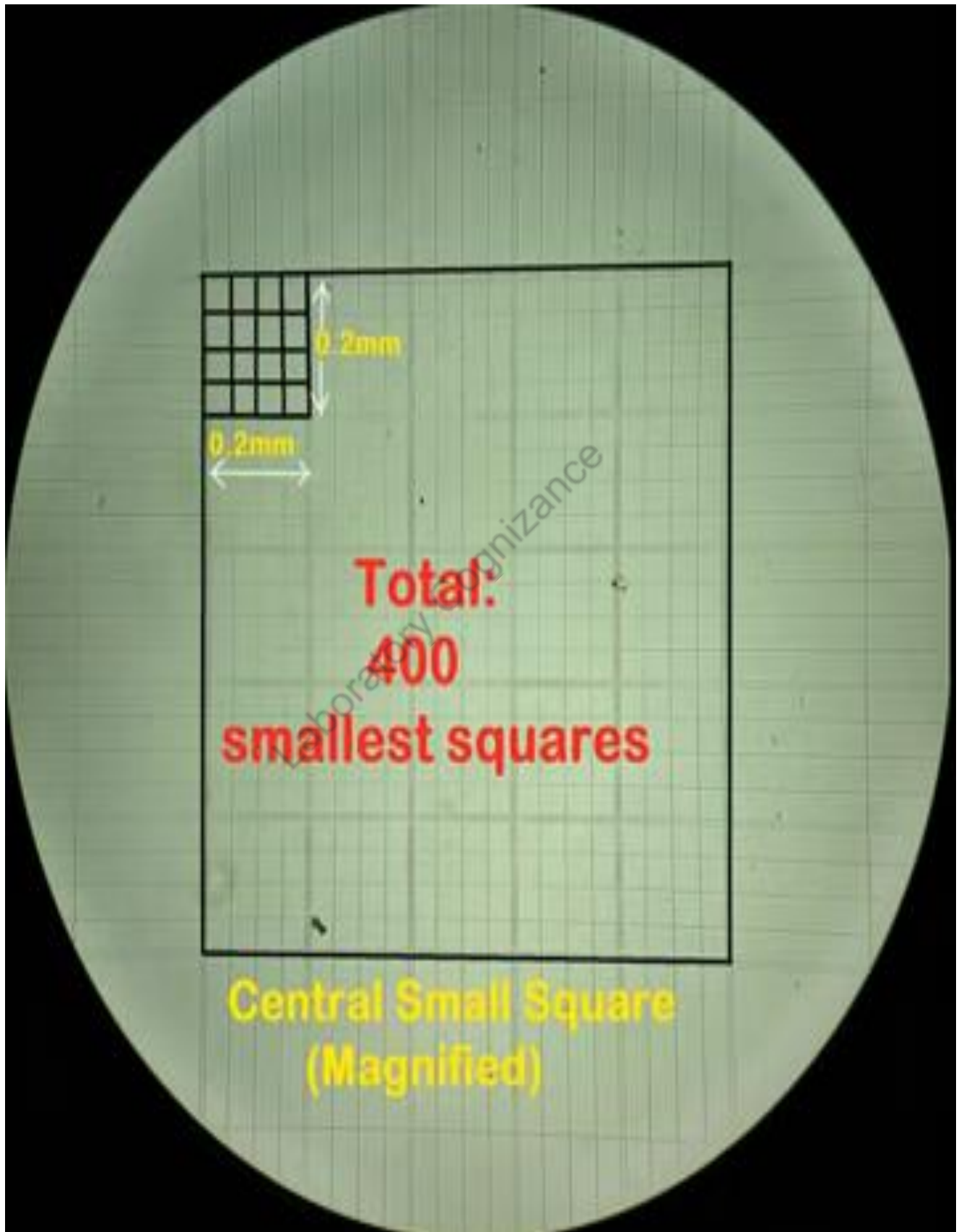
- Where each square (25 smaller squares) measuring 0.2×0.2 mm, with an area of 0.04 square millimeters.



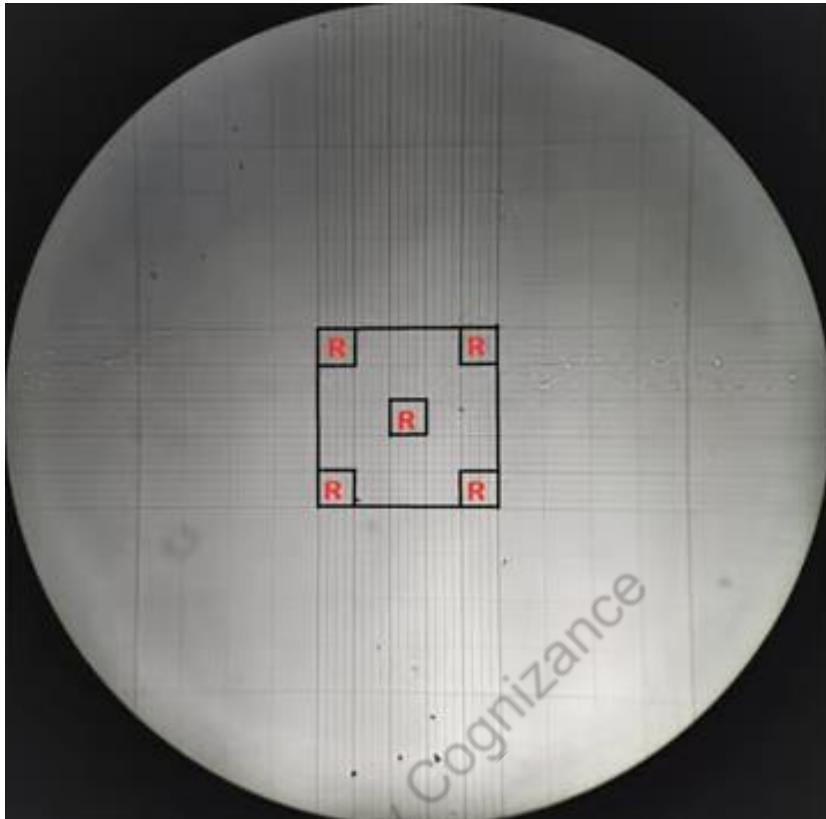
- Each of these smaller squares is further subdivided into 16 smallest squares. Each of these 16 smallest squares have a dimension of 0.05×0.05 mm, with an area of 0.0025 sq. mm.



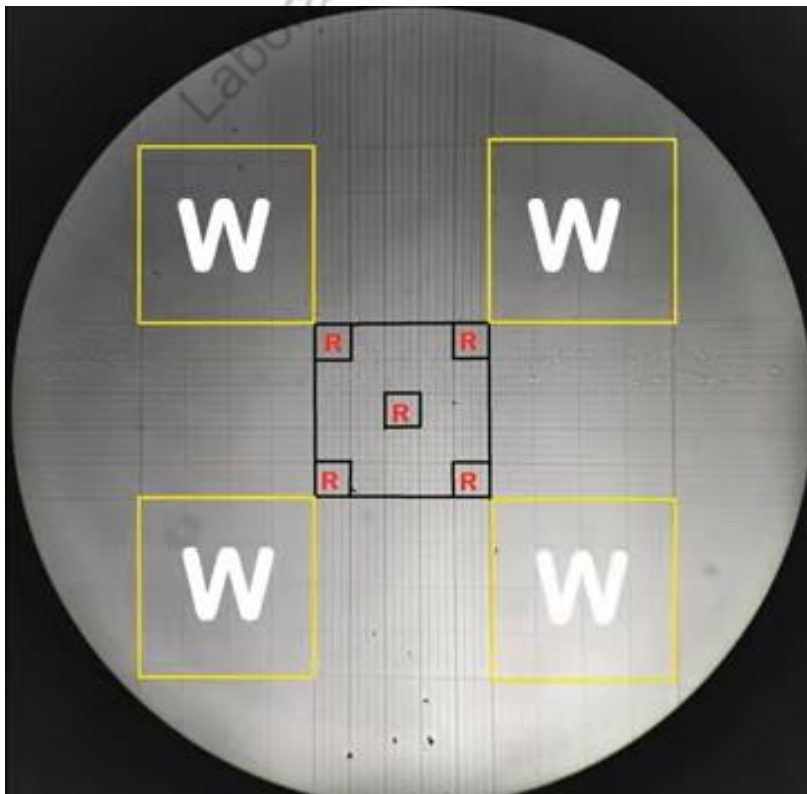
- The central small square (that we use for RBC-count) therefore comprises of a total of 400 smallest squares



- Thus, volume occupied by different squares (singularly or wholly) can easily be worked out using data on their dimensions & depth of the counting chamber.
- The area in the central small square marked "R" used for counting RBC.

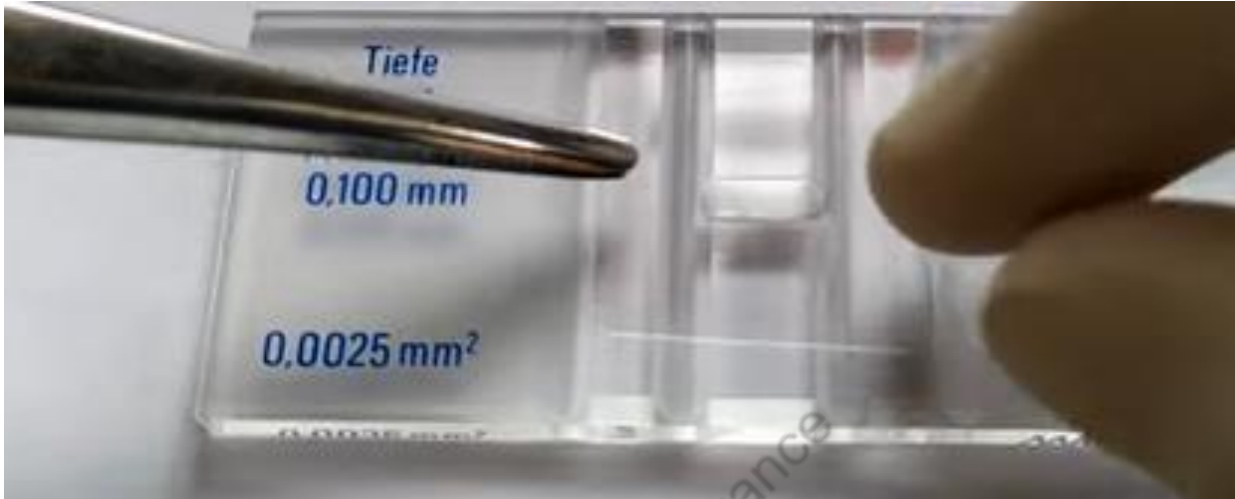


- While four small corner squares marked "W" are used to count white blood cells.

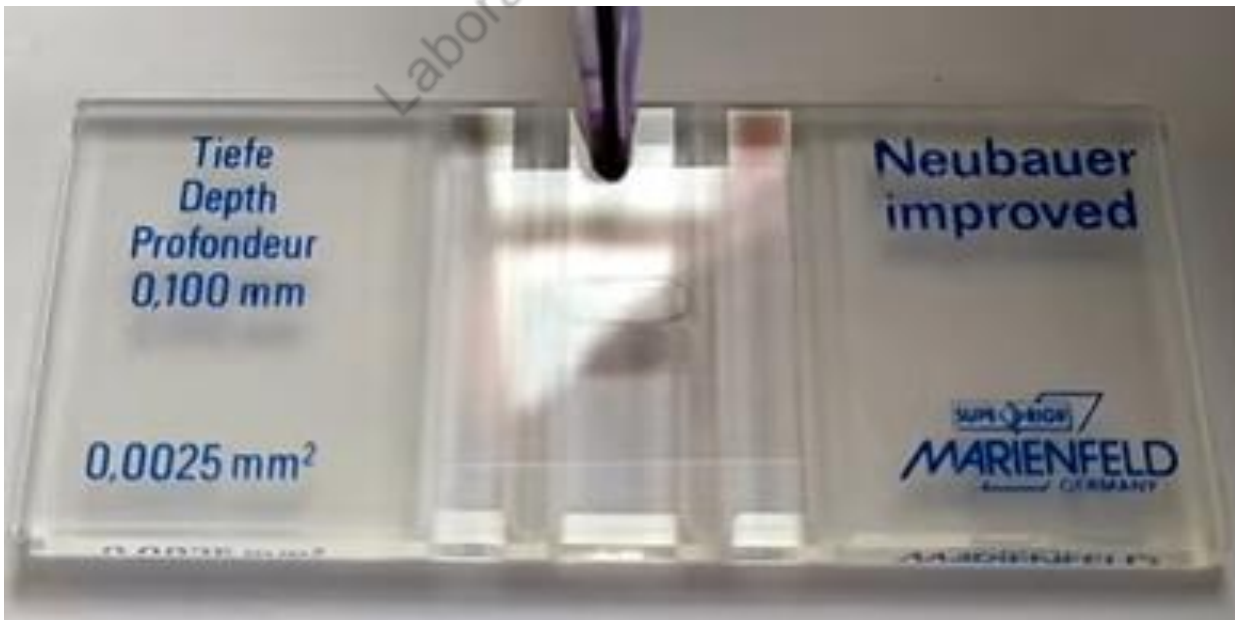


Counting cells with Haemocytometer or Haemacytometer

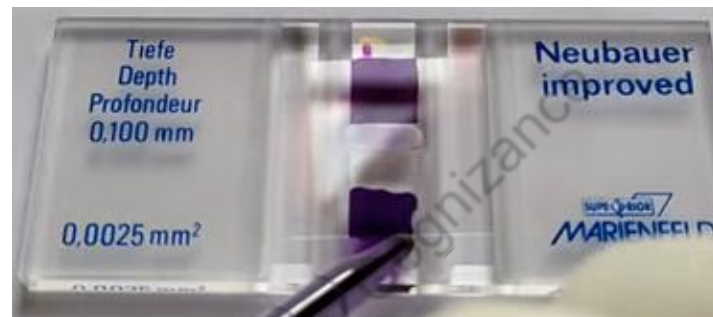
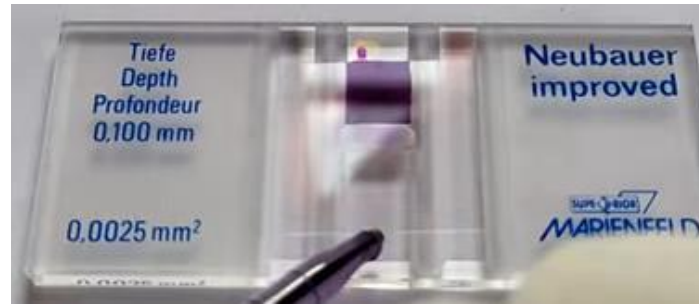
- Prepare Haemocytometer & clean it (chamber surface) properly with ethanol 70% or distilled water, then wipe dry with a tissue. Using 45-degree angle, Position the coverslip over the chamber, making sure that it does not move.



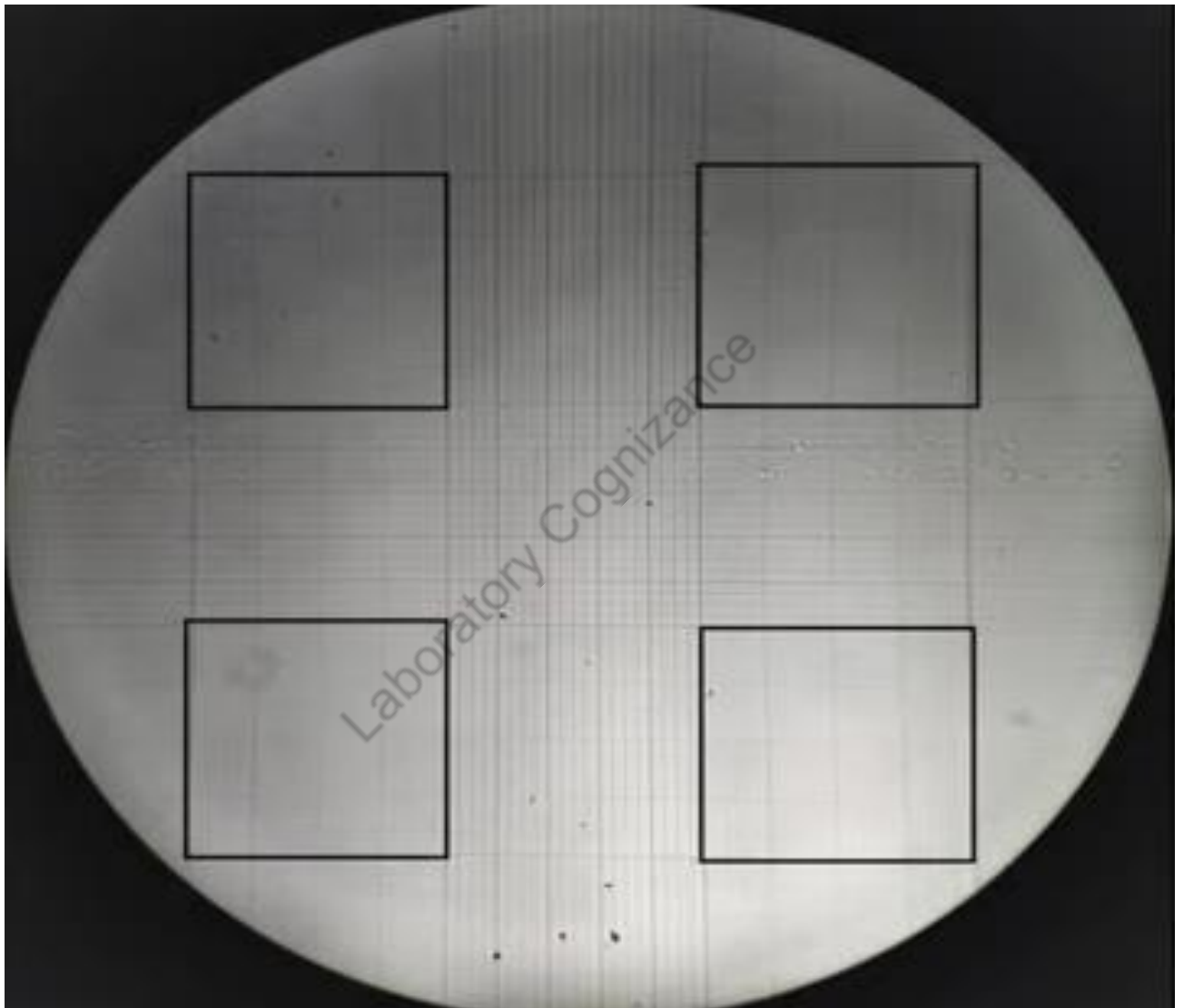
- Mix spore sol. Well, add 10 μ l of spore sol. to each side of the haemocytometer (carefully position the tip of the pipette on one edge of cover glass, allow the content of the pipette to gradually flow into the narrow space b/w the cover glass & the counting chamber via capillary force, this action is known as **Capillary Reaction**)



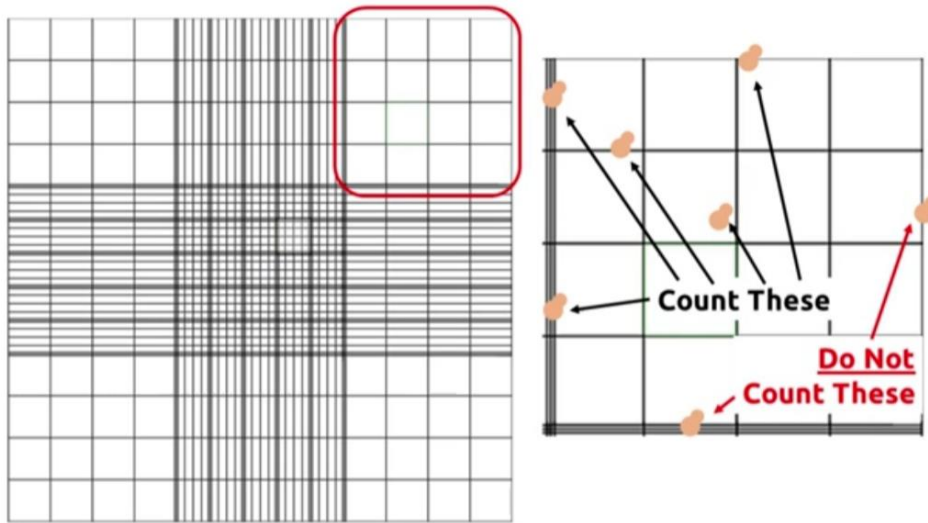
- Observing capillary reaction's flow, one may load the sample to charge the other side of counting chamber as well, when the space of counting grid is filled, one is done with that side, remember not to over fill the chamber. (Carefully observe the below images)



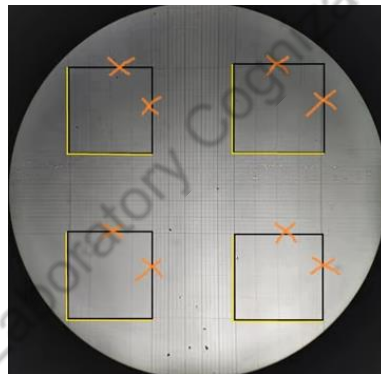
- Keep a side counting chamber to allow few minutes to spore suspension to settle within the slide.
- After that slide is now ready for microscopic observation.
- First locate the small square (central one) at the four corners of the counting grid under low power of microscope. Switch to 10x to 40x objectives to begin the counting.
- One may also need to adjust the light intensity of the microscope to visualize the fine lines in the counting grid.



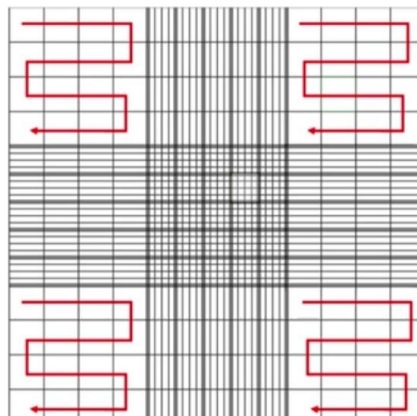
- Applying the **L-Rule** of the counting of cells or spores.



- Count the cells lying inside of these squares & those lying on border of left side or bottom(lower) side of each square.
- Do not count those cells lying on the borders of the upper & right sides of the square

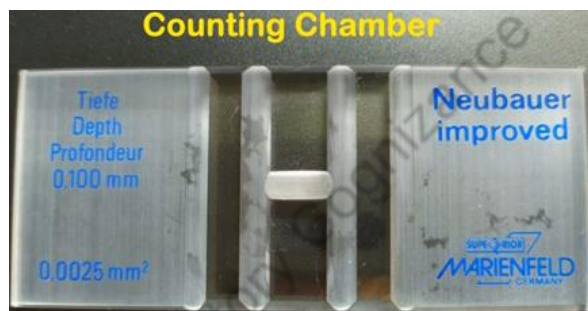
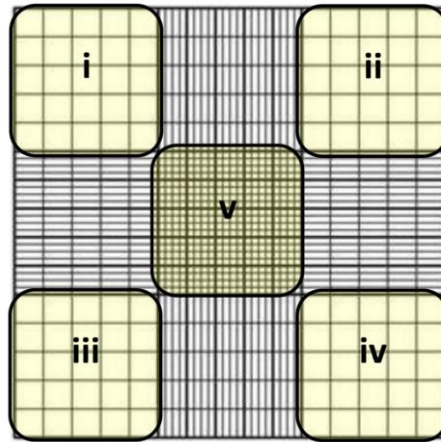


- Count cells in a zig-zag manner.



What to Count in sense of Spores

- Count the spores in small squares (see the below image) (i, ii, iii, iv, and v) on both sides of the hemacytometer. Write down the numbers & find the average.



Experiment of Spores Density (number of spores/ml)

- This experiment is about measuring the number of spores per millilitre (ml) in a specific solution.
- Spore density means how many spores are in a certain amount of liquid. Spores are tiny particles that can grow into plants or bacteria. Spore density is important for scientists who study plants & diseases because it helps them do their experiments correctly. It also helps them know if there are too many spores in a sample.
- To find spore density, scientists use a special tool & a microscope to count the spores in a small amount of liquid. Sometimes, they might need to make the liquid weaker to see the spores better. When they know how many spores are in the liquid, they can learn useful things for their research.
- So, spore density is like counting how many tiny particles are in a drop of liquid. This helps scientists do better studies & understand things better.

The process of measuring spore density with a focus on dilution preparation when the spore density is too high for accurate counting:

Collecting Sample: Get a sample of the spores from the fungus on the petri dish. Use a clean pipette or loop to gently get the spores.

Making it Weaker: If there are too many spores in the sample, it's hard to count them accurately. So, make it weaker by mixing the sample with some liquid. How much you mix depends on how strong the spores are at the start & how many you want to count.

Mix the Weaker Sample: Mix the weaker sample really well so the spores are spread out evenly.

Counting Spores: Take a small bit of the weaker spore mix & put it on a special plate made for counting things. Wait a bit so the spores spread out evenly on the plate.

Look and Count: Put the plate under a microscope and use the right magnification to see the spores clearly. Count how many spores are in a certain area using the squares on the plate.

Find the Amount: Figure out how many spores there are in the weaker sample. You use a formula for this:

$$\text{Spore Amount (spores/ml)} = (\text{Number of spores you counted} / \text{Amount of weaker sample})$$

If you know how much weaker you made the sample, you change the formula a bit:

$$\text{Spore Amount (spores/ml)} = (\text{Number of spores you counted} / \text{Amount of weaker sample}) \times \text{How much weaker you made it}$$

Change for the Weaker Sample: Since you made the sample weaker before, you need to fix the final amount. If you checked the spores after making it weaker, you need to change your final number by how much weaker you made it.

This way, you can find out how many spores are in a small bit of liquid. It helps scientists do better research & learn more.

Prepare a Spore Suspension

Get the things you need: Get the spore sample, something to mix the spores with (like clean water or nutrient broth), & a container to mix them in.

Measure the spores: Figure out how many spores you want to use. You can think about how much you have, either in drops (like 1 drop) or how many spores there are (like 1 million spores).

Make the mixing liquid: In a different container, make the mixing liquid weaker according to a plan. For example, if you want to make it 100 times weaker, mix 1 part of the spore sample with 99 parts of the mixing liquid (like 1 drop of spores + 99 drops of liquid).

Mix the spores and liquid: Put the spores you measured into the container with the liquid. Mix them well by shaking the container gently.

Let the spores spread: Wait for a bit so the spores spread out evenly in the liquid.

Put a label and keep it safe: Write on the container what kind of spores they are, how much weaker you made the mix, & the date. Keep the container in the right place (like a certain temperature or darkness) until you're ready for the next steps of your experiment.

Remember, it's important to be careful and clean while doing this. You want to make sure your spores stay clean & right for your experiment.

Counting Spores with Marienfeld's Haemocytometer in a Clean Workspace (LAF)

Goal: Count spores in a spore mix correctly using Marienfeld's haemocytometer in a clean workspace. This keeps things sterile and avoids any dirt getting in.

Stuff You Need:

- Marienfeld's haemocytometer.
- Spore mix that's 1:100 (100 ml).
- Clean, throwaway tips+pipettes.
- Microscope.
- Clean workspace without dust (LAF).

Steps:

Step 1: Make a Spore Mix

Inside the clean workspace (LAF), in a place without dust, make a spore mix that's weaker. This means using 100 ml of the 1:100 spore mix. Before you start, clean everything to keep things clean & germ-free.

Step 2: Put Spores on haemocytometer

Use clean, throwaway pipettes to put 10 μ l of the weaker spore mix on each square of Marienfeld's haemocytometer. Do this in the clean workspace to keep it sterile.

Step 3: Wait for Spores to Settle

Let the spores settle on the haemocytometer squares for a little bit. This helps them spread out evenly. Keep the haemocytometer in the clean workspace while you wait.

Step 4: Look with Microscope

Use a microscope in the clean workspace to look at & count the cells in each square of both grids on the haemocytometer.

Step 5: Find Average Cell Count

Count the cells in both grids, keeping everything clean. Find the average count by adding the counts from both grids and then dividing by the number of grids. For example, Suppose, if you counted 100 cells in the first grid & 90 cells in the second grid, the average would be 95 cells.

Step 6: Find Total Cells

With the average cell count, the dilution factor (1:100), and the total volume of the spore mix (100 ml), figure out how many cells are in the whole spore mix:

$$\text{Total Cells} = \text{Average Cell Count} \times \text{Dilution Factor} \times \text{Total Volume of Spore Mix}$$
$$\text{Total Cells} = 95 \text{ cells} \times 100 \times 100 \text{ ml} = 950,000 \text{ cells}$$

The dilution factor is calculated as:

$$\text{Dilution Factor} = \text{Total Volume of Final Solution} / \text{Volume of Original Substance}$$

$$\text{Dilution Factor} = 100 \text{ ml} / 1 \text{ ml}$$

$$\text{Dilution Factor} = 100.$$

Step 7: Change Cells to Volume (ml) of Spore Mix

Calculate the volume of the spore mix in millilitres (ml) using the total cell count and the volume you used for counting (10 µl):

$$\text{Volume of Spore Mix (ml)} = \text{Total Cells} / \text{Number of Cells Counted} \times \text{Volume Used for Counting}$$

$$\text{Volume of Spore Mix (ml)} = 950,000 \text{ cells} / 190 \text{ cells} \times 10 \text{ µl} = 50,000 \text{ µl}$$

Step 8: Change Volume to ml

Change the volume from microliters (µl) to millilitres (ml):

$$\text{Volume of Spore Mix (ml)} = 50,000 \text{ µl} / 1,000 = 50 \text{ ml}$$

Step 9: Write Down Results

The whole cell count in the spore mix is 950,000 cells, & the volume of the spore mix you used for counting is 50 ml. You did everything in a clean workspace to keep things germ-free.

Final thought: By doing this method in a clean workspace, you can count spores the right way in the lab. Marienfeld's haemocytometer helps you count cells & work out how many there are accurately.

To find the spore density (number of spores per millilitre), use the following formula:

$$\text{Spore Density} = \text{Total Cell Count} / \text{Volume Used for Counting}$$

For this specific case:

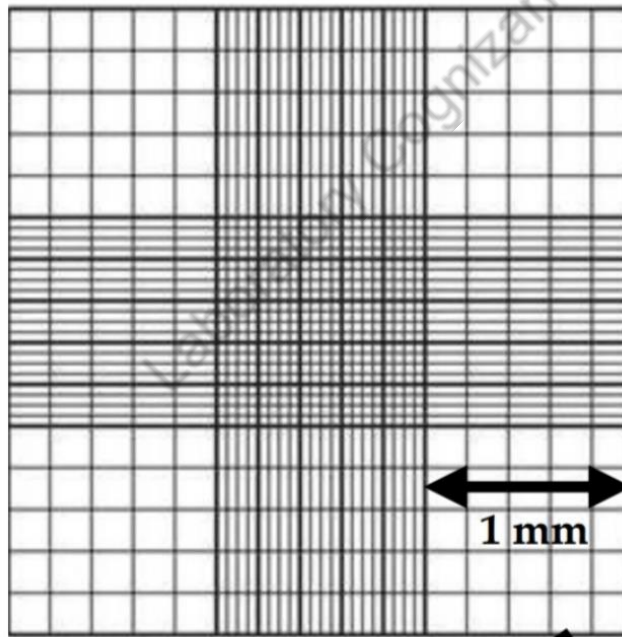
- Spore Density = 950,000 cells / 50 ml
- Spore Density = 19,000 cells/ml

In this scenario, the calculated spore density is 19,000 spores per millilitre (spores/ml) of the spore suspension that was measured using the haemocytometer.

For Quick and Easy Calculation of Spores

- To do Haemocytometer calculation without using any formula, just remember this:

100 nl (nanolitres)



Note box:

Area of large square = 1 mm x 1 mm = 1 mm²

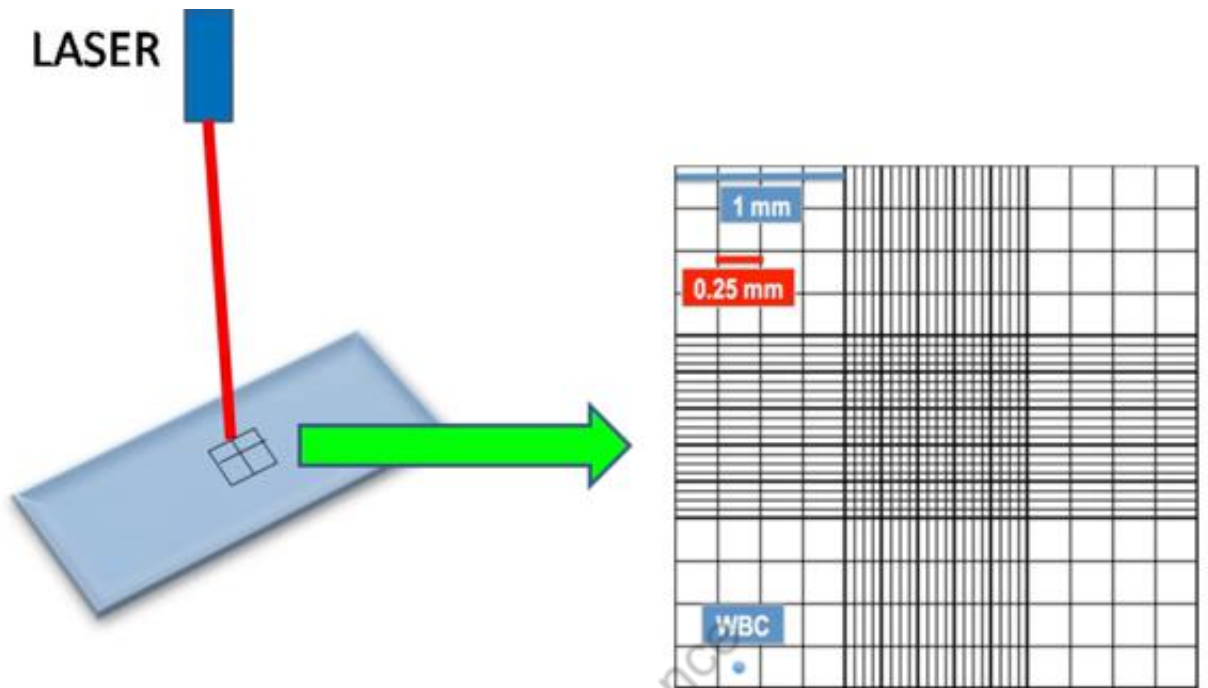
Depth of haemocytometer view side: 0.1 mm

Volume = Depth x Area

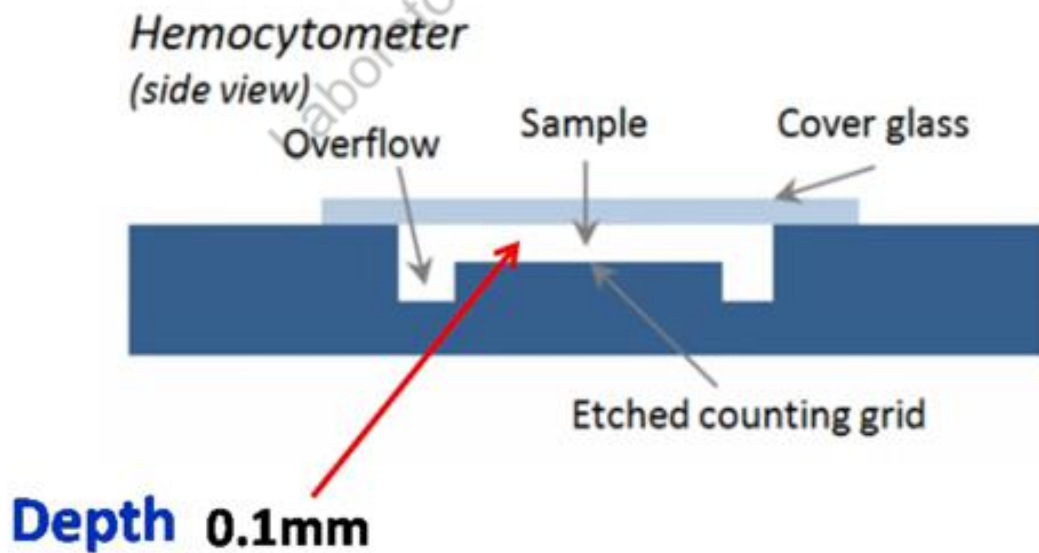
⇒ Volume = 0.1 mm x 1 mm² = 0.1 mm³

⇒ 0.1 mm³ = 0.1 µl = **100 nl**

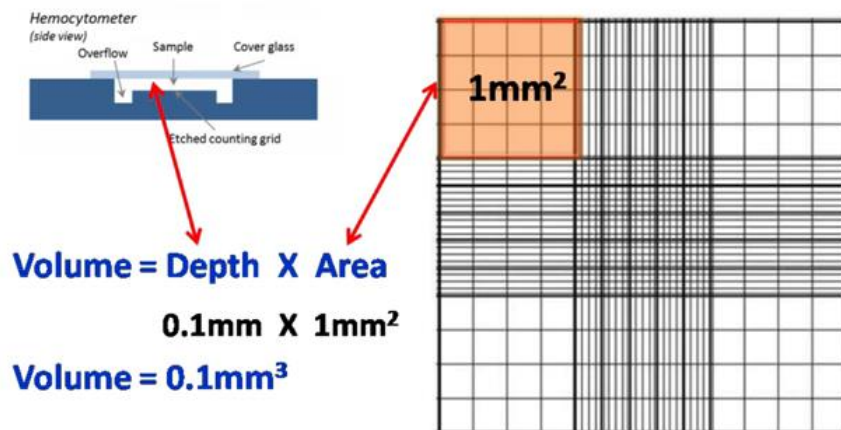
- The glass of haemocytometer is carefully crafted with a laser, such that the area (the square) bounded by the line is known.



- Depth of the chamber is 0.1mm.

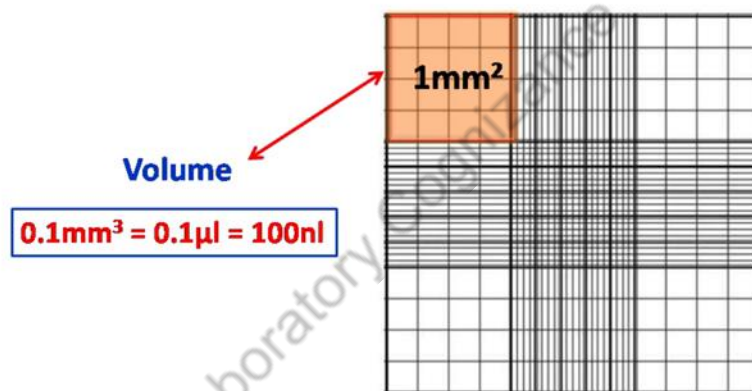


- Now, if we multiply the area with its depth than one gets the volume.

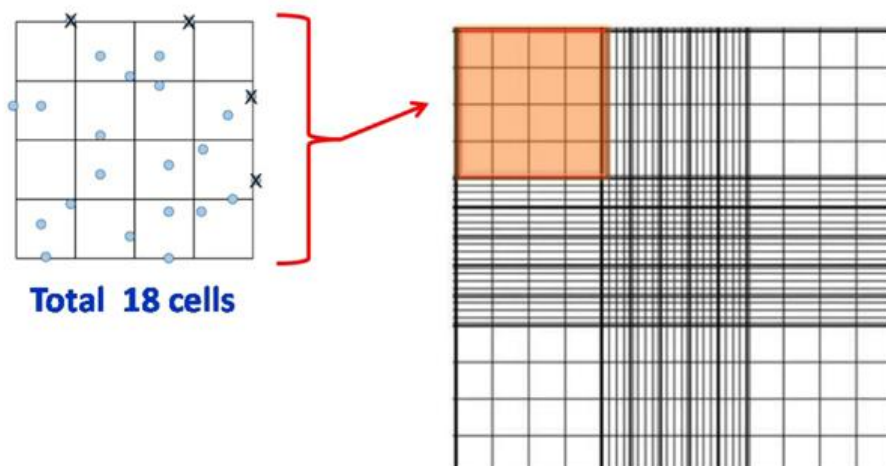


$0.1 \text{ mm}^3 = 0.1 \mu\text{L} = 100\text{nl}$

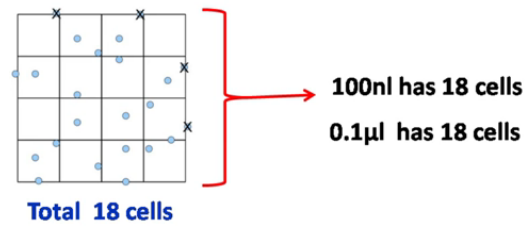
- The volume occupied by each of the 09 squares (small) is around 100 nano Liters.



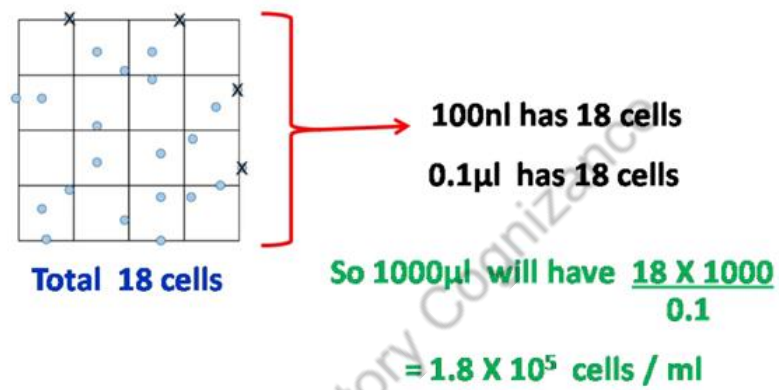
- Suppose total number of cells that one has calculated in the following small square (one of 09) is 18, as shown in below image.



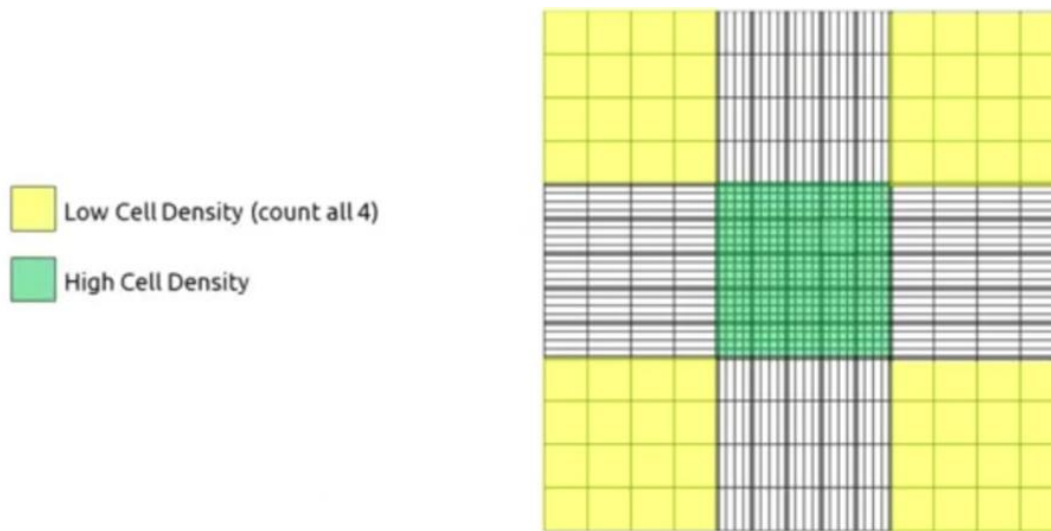
- So, this means that 100 nano Liters has 18 cells, or one can say that 0.1 microliter has 18 cells.



- So, the total number of cells in thousand micro-Liters. that is 1-ml is going to be 1.8×10^5 .



Measurement of Spore Density (No. Of Fungal Spores in 1 – ml of Spore Suspension)

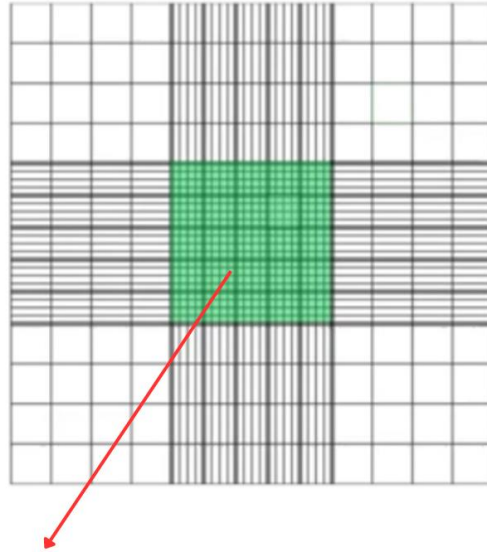


Things you need:

- Fungal culture without any contaminants
- Small surgical knife
- Small tube (Eppendorf tube)
- Very small pipette (measuring 1000 microliters, which is like 1 millilitre)
- Special dye called Lactophenol Cotton Blue (used to make spores easier to see)
- Special slide called a haemocytometer (used to count things)

Steps to follow:

- Start by taking a tiny amount (1 millilitre) of clean water & carefully putting it into a small tube called an Eppendorf tube. You'll need a very small pipette for this, which can measure 1000 tiny drops.
- Use a small knife or a loop to collect spores from the fungal culture on a special plate. Put these spores into the small tube with the water.
- Close the tube and gently shake it to make sure the spores spread out evenly in the water. You can use a special tool called a Vortex to mix them well before you put the mixture on the slide.
- If the spores don't have much color, you can add a special dye called Lactophenol Cotton Blue to the mixture. This dye makes the spores easier to see. If the spores are already colourful, you don't need to do this step.
- Use a pipette to take out 10 microliter or let's say for understanding that tiny drops of the spore mixture & put them onto a special slide with counting areas or grids.
- Wait a little bit to let the spores settle in the grids of the slide. Then, you can use a microscope to look at the slide & count the spores.
- By doing these steps, you'll be able to count and study the spores in the water mixture accurately.



Depth = 0.1mm

(L x B) Area = 0.0025 mm²

Volume or Capacity = Depth X Area

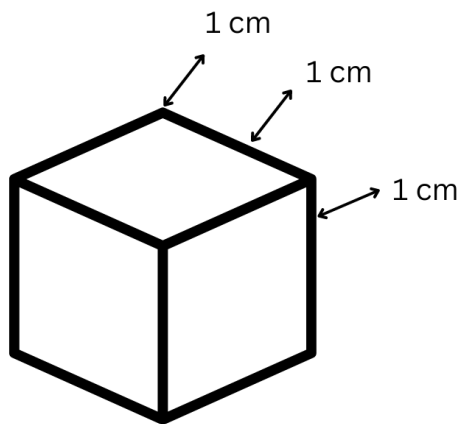
V = 0.00025 mm³

- Count the spores of each cell & take the average of them, to get the “n” value.

Number of spores in 1ml = $n/v \times 1 \text{ ml}$

Number of spores in 1ml = $N/0.00025\text{mm}^3 \times 1 \text{ ml}$

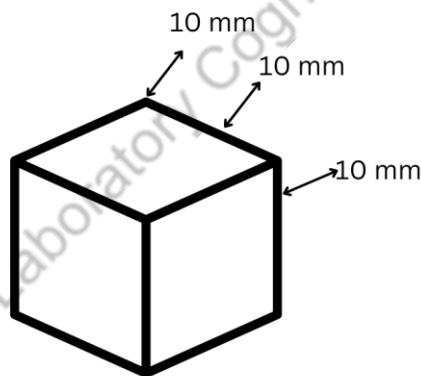
- Focusing on above formula, Let's covert 1ml into millimeter cube.
- To understand this let's consider a cube's example.



$$l \times b \times h = 1 \text{ cm} \times 1 \text{ cm} \times 1 \text{ cm} = 1 \text{ Cubic Centimeter (cm}^3\text{)}$$

Or Let's say 1 cc

$$1 \text{ cc} = 1 \text{ ml}$$



$$l \times b \times h = 10 \text{ mm} \times 10 \text{ mm} \times 10 \text{ mm} = 1000 \text{ Cubic Millimeter (mm}^3\text{)}$$

$$\text{As, } 10 \text{ mm} = 1 \text{ cm}$$

- So, the above formula can be written as:

$$\text{Number of spores in 1ml} = n/v \times 1000 \text{ mm}^3$$

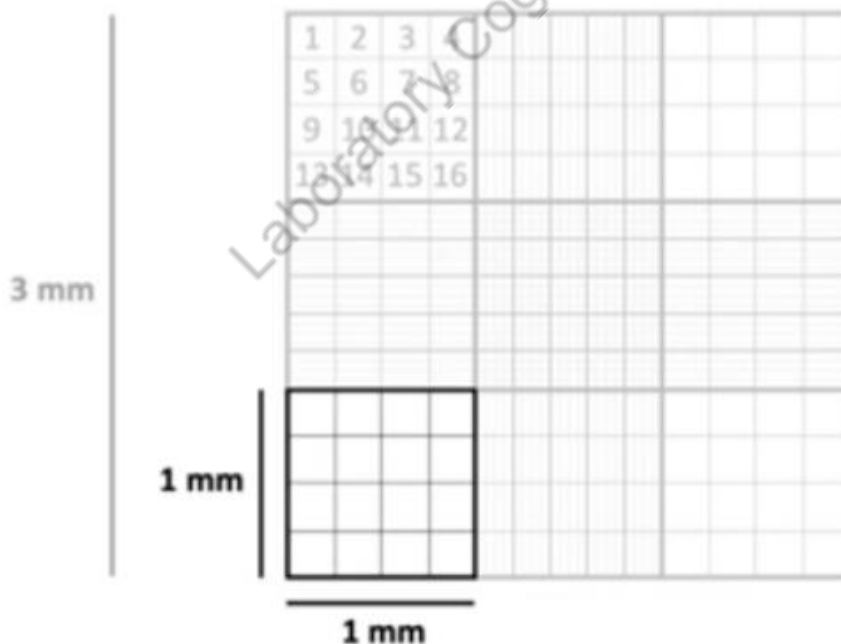
$$\text{Number of spores in 1ml} = N/0.00025 \text{ mm}^3 \times 1000 \text{ mm}^3$$

- So, by applying & calculating those values in above formula, one can simply calculate the spores/ml.

Exploring Spore Density per ml: A Practical Introduction

Some Points to understand

- If one has calculated the spore count per millilitre (ml) of a spore suspension using a haemocytometer, then the count indeed be considered the spore density per ml.
- When counting fungal spores using a haemocytometer, it's common to use the 4 corner squares (which have 16 smaller squares in each) for counting. This is like the method used for counting white blood cells. The middle section, as used for red blood cell counting, might not be suitable for accurately counting fungal spores due to its larger size and different grid pattern. So, focus on the 4 corner squares for your fungal spore counting.
- If you take the 1 ml spore suspension you've prepared & then add it to 9 ml of distilled water, you will achieve a 1:10 dilution. This means you'll have a total of 10 ml of solution, with 1 ml being spore suspension & 9 ml being distilled water, resulting in a dilution factor of 10.
- The below image illustrates those one of the nine big squares that are used for counting cells inside has a size of 1 time 1 millimetre resulting in a surface area of 1 square millimetre.



- Since three-dimensional solution is applied to the slide then the height is also an important parameter to calculate the volume of the sample.
- Remember in Neubauer Counting Chamber the distance b/w slide & the coverslip is 0.1 mm.
- So, in total that means that one of those 9 squares contains a volume of 0.1 microliter.

Preparing the Haemocytometer:

- Clean the haemocytometer and coverslip thoroughly to ensure there's no contamination.
- Place the coverslip on the haemocytometer, aligning it with the grid lines.

Preparing the Spore Suspension:

- Mix the fungal spore suspension well to ensure an even distribution of spores.
- If desired, add a small amount of trypan blue or other staining solution to the spore suspension. This can help in distinguishing live spores from debris.

Loading the Haemocytometer:

- Using a 1 ml pipette, carefully load the spore suspension into the space between the coverslip and the haemocytometer.
- Allow the suspension to fill the grid area through capillary action.

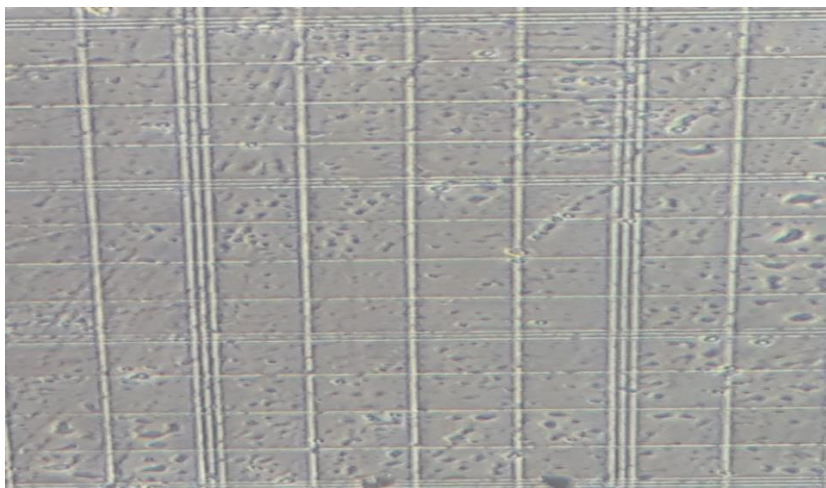
Microscopic Examination:

- Place the loaded haemocytometer on the microscope stage and adjust the focus to view the grid lines and spores.
- Choose a representative grid area for counting. This could be a central grid or an edge grid.
- Using a high-power objective, count the number of spores in the chosen grid area. Count only those spores that touch the top and left boundary lines of the grid, but not the bottom and right boundary lines. This avoids duplication of counting at the edges.
- Repeat the counting process for several grid areas to get an average count.

Calculation:

Calculating the Average Spores in Each Grid Area:

- To find the average number of spores in one grid area, which we'll call "n," follow these steps:
- Look at the four corner squares of the grid.
- Count the total number of fungal spores in these four squares.
- Divide this total by 4 to get the average number of spores per corner square.
- This average number of spores per corner square is what we'll call "n".



Simplest Out of the Box Example for Understanding Fungal Spore Calculation:

Suppose;

- **Counted Spores (Average Value):** 161 Spores.
- **Counted Area: four squares:** ($= 4 \times 1 \text{ mm}^2$) = 4 mm^2 .
- **Chamber's Depth:** 0.1 mm.
- **Spore Suspension=** 1ml.

$$\text{Volume} = \text{Depth} \times \text{Area}$$

$$V = 0.1 \text{ mm} \times 4 \text{ mm}^2$$

$$V = 0.4 \text{ mm}^3$$

$$\text{Number of spores in 1ml} = n/v \times 1000 \text{ mm}^3$$

$$\text{Number of spores in 1ml} = 161/0.4 \text{ mm}^3 \times 1000 \text{ mm}^3$$

$$\text{Number of spores in 1ml} = 402500 \text{ spores/ml Answer}$$

diluting the spore solution to reach the desired concentration of spores per millilitre (spores/mL)

One can achieve this by employing the formula:

$$C1V1 = C2V2$$

Example,

If For instance, if you have a spore solution measuring 402500 spores/mL & your target is to have 40 mL of a solution containing 50,000 spores/mL

Data:

- **C1:** 402500 spores/ml
- **V1:** X
- **C2:** 50,000 spores/ml
- **V2:** 40 ml

Putting the Values directly into the above formula:

$$402500 \times (x) = 50,000 \times 40$$

So,

$$X = \frac{50,000 \times 40}{402500}$$

So,

$$X = 4.96 \sim = 5 \text{ ml.}$$

So,

So, one will add 5mL spore solution to 35mL sterile dH2O (40-5=35) to give a final concentration of 50,000 spores/mL in 40mL

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