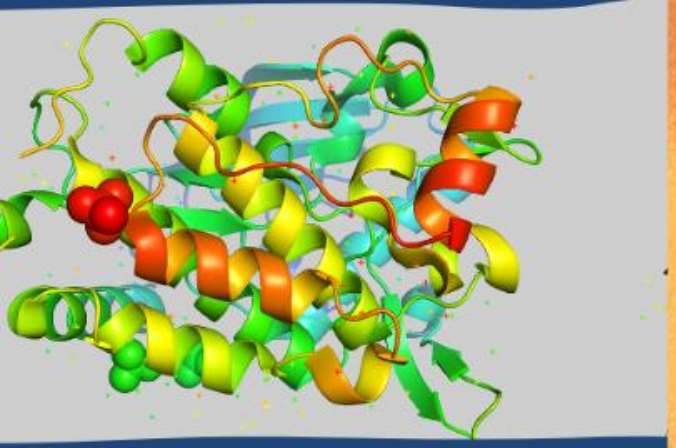
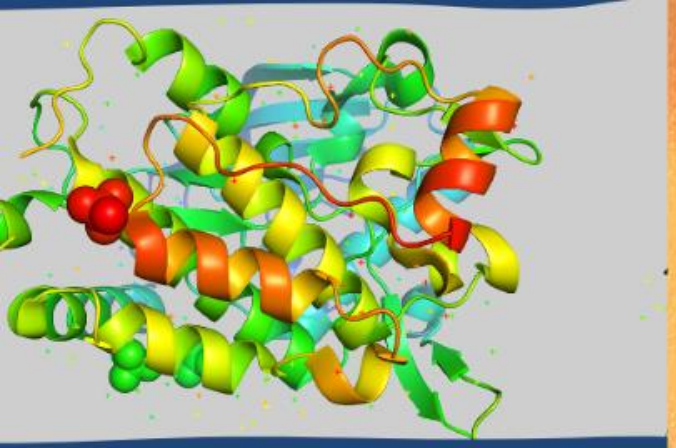
Impact of anti-fungal medication on cell growth



Aim

To investigate the effect of tea tree oil on the growth of *S. cerevisiae*.

- Investigate the effect of a natural anti-fungal medication on the growth of *S. cerevisiae*.
- This could be adapted to investigate antimicrobial agents on bacterial growth, e.g. using *E. coli strains B* or *K12*.
- The effects of the antimicrobial agent can be measured using a colorimeter (indirect) or a haemocytometer (direct).



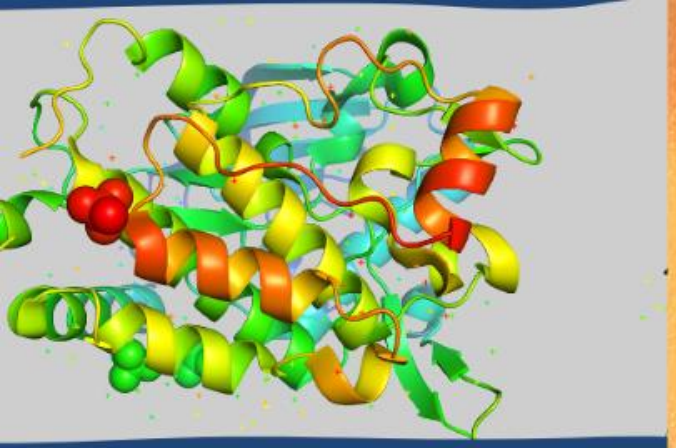
Curriculum

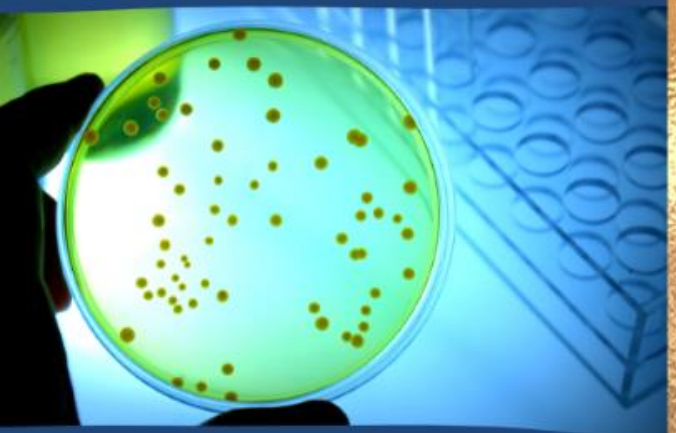
(e)Microscopy
Bright-field microscopy is commonly used to observe whole organisms, parts of organisms, thin sections of dissected tissue or individual cells

Unit 1, KA1e

Method and use of haemocytometer to estimate cell numbers in a liquid culture

Unit 1, KA1f

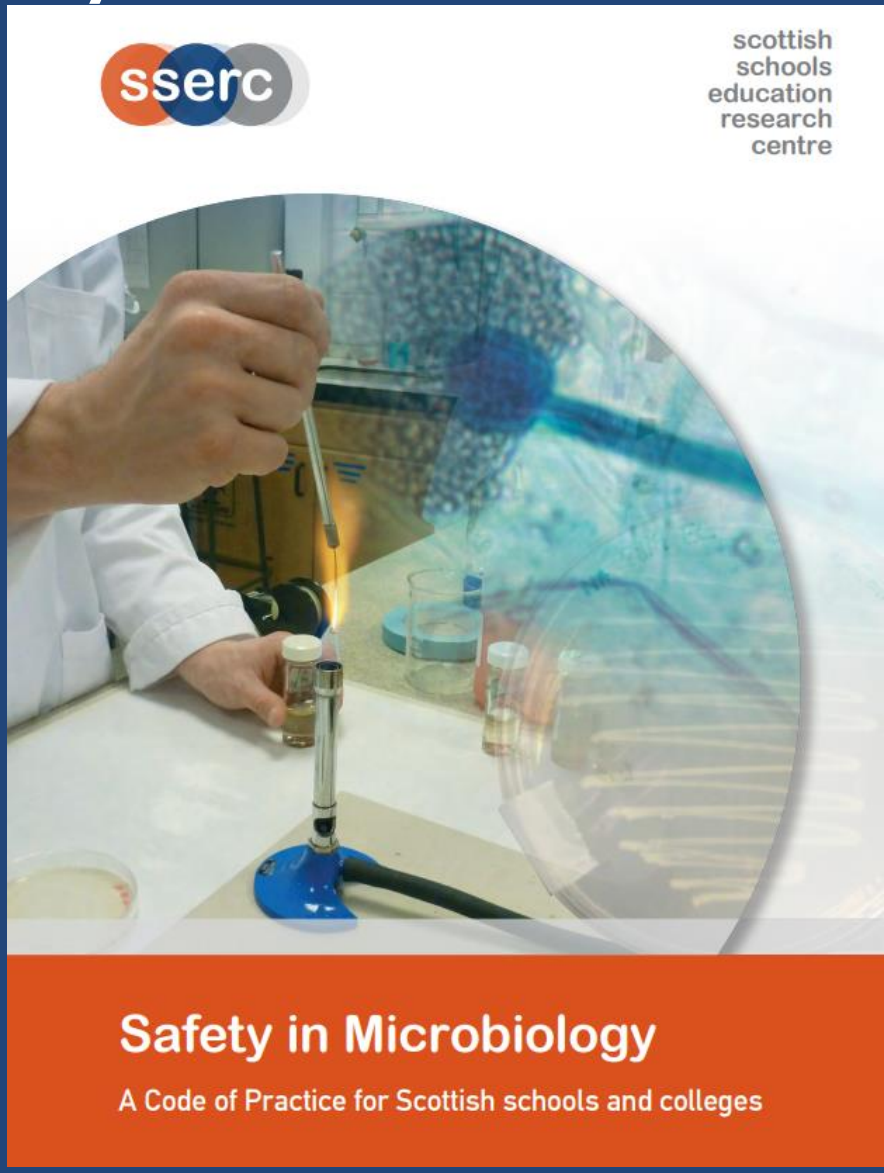


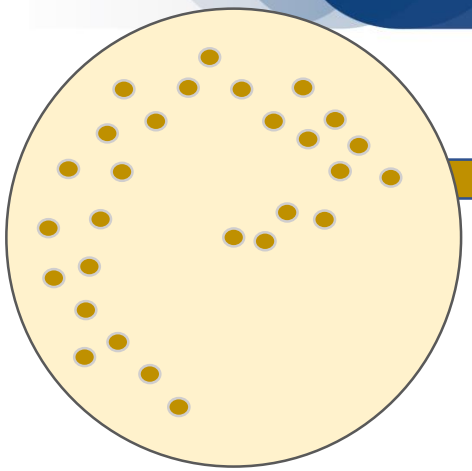


Health & Safety

SSERC Code of Practice – Safety in Microbiology.

A Level 3-trained technician must be involved in the culturing of the microorganisms to be assured of aseptic technique and that cultures are free from contamination prior to opening.





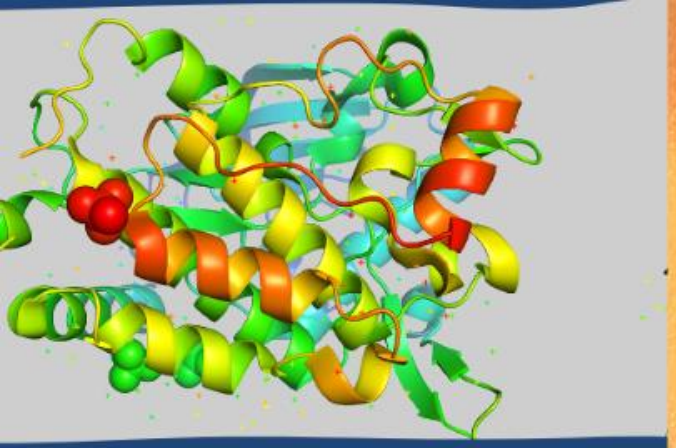
YGA streak plate of *S. cerevisiae*

Inoculate YGB with a single colony

YGB +/- anti-fungal medication

Incubate for 12-24 hours at 30°C.

Perform a direct cell count using a haemocytometer.



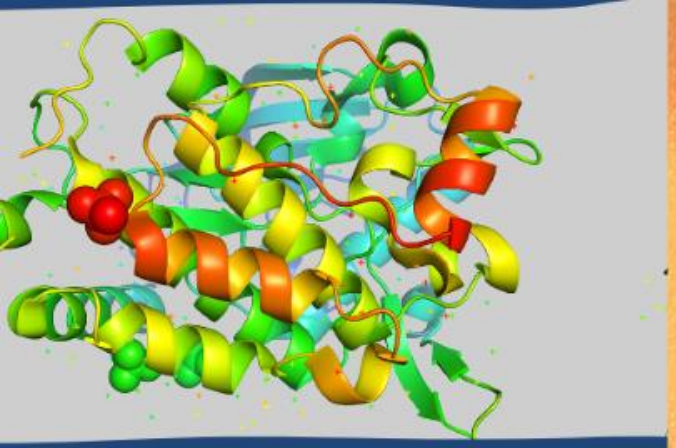
Organism - rationale

S. cerevisiae

- This organism ensures that low risk is presented if cultures are spilled.

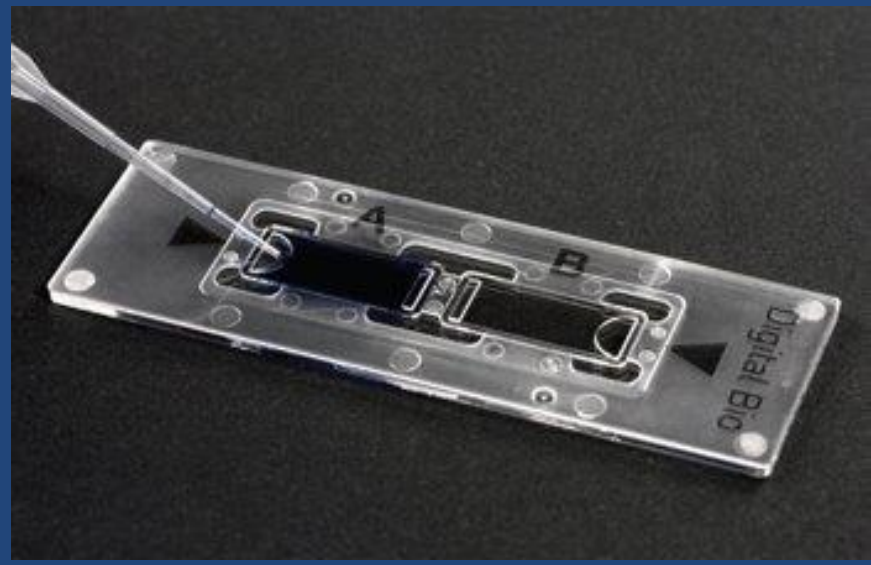
- *S. cerevisiae* is a low-risk organism.

- However, a level 3 trained person is required to oversee the work to minimise risk of potential contaminants.



Materials

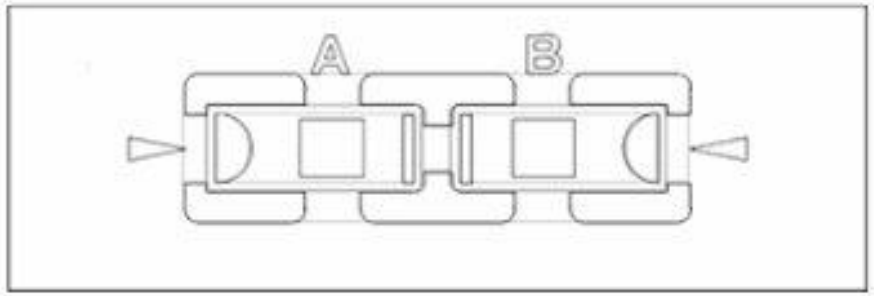
- *S. cerevisiae* grown overnight ± tea tree oil
- Pipettes
- Discard jar
- C-Chip haemocytometer
- Light microscope
- Methylene blue

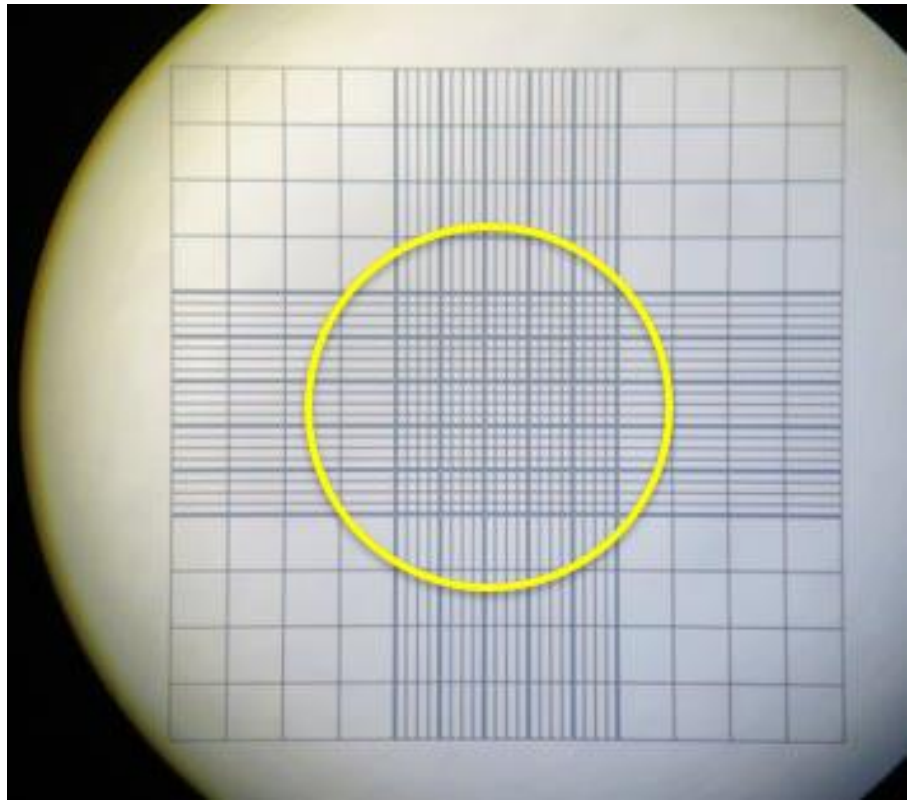


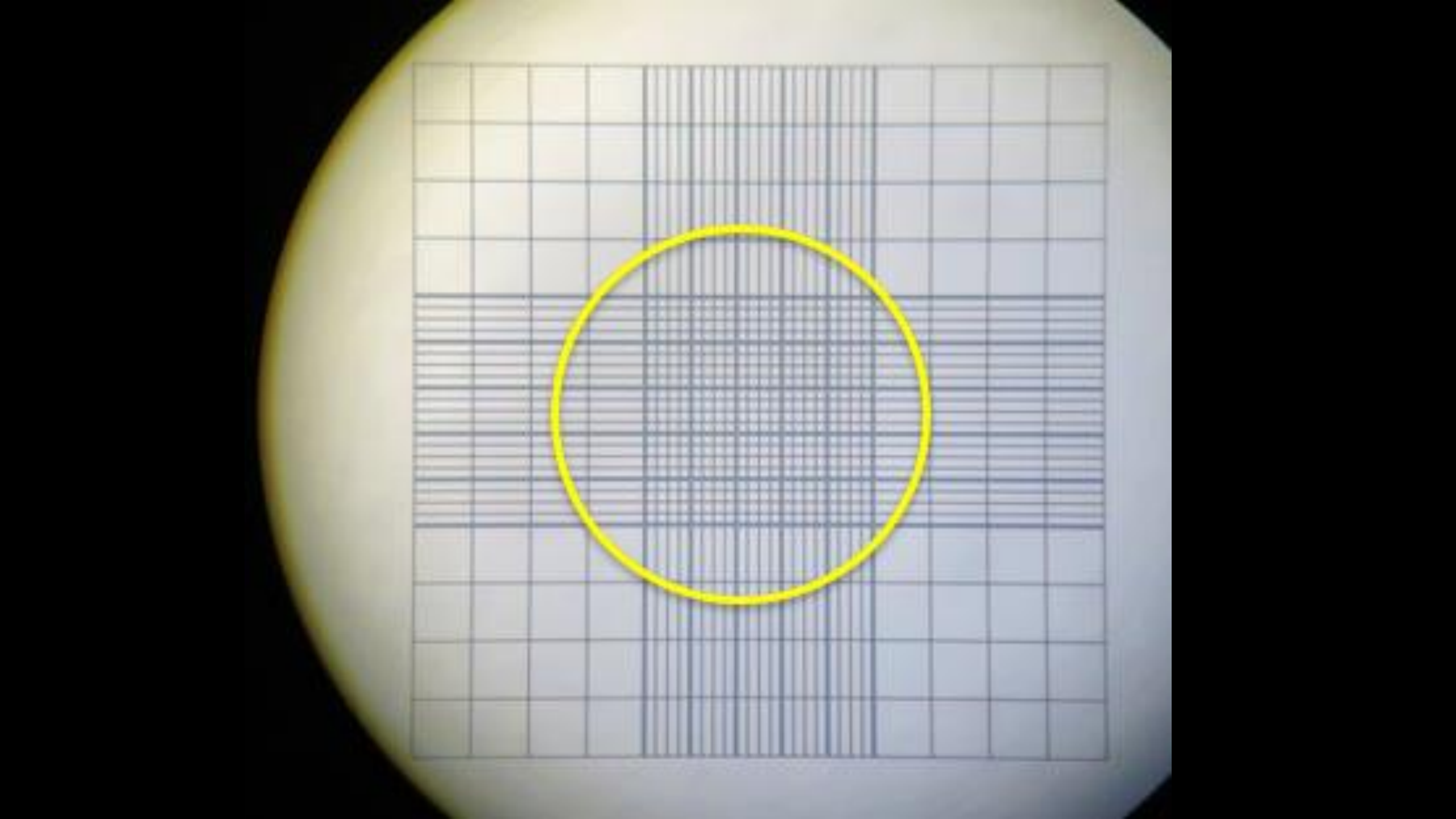
Method

Place the C-Chip haemocytometer on the stage of the light microscope.

Using the x4 objective lens, locate the central counting grid.

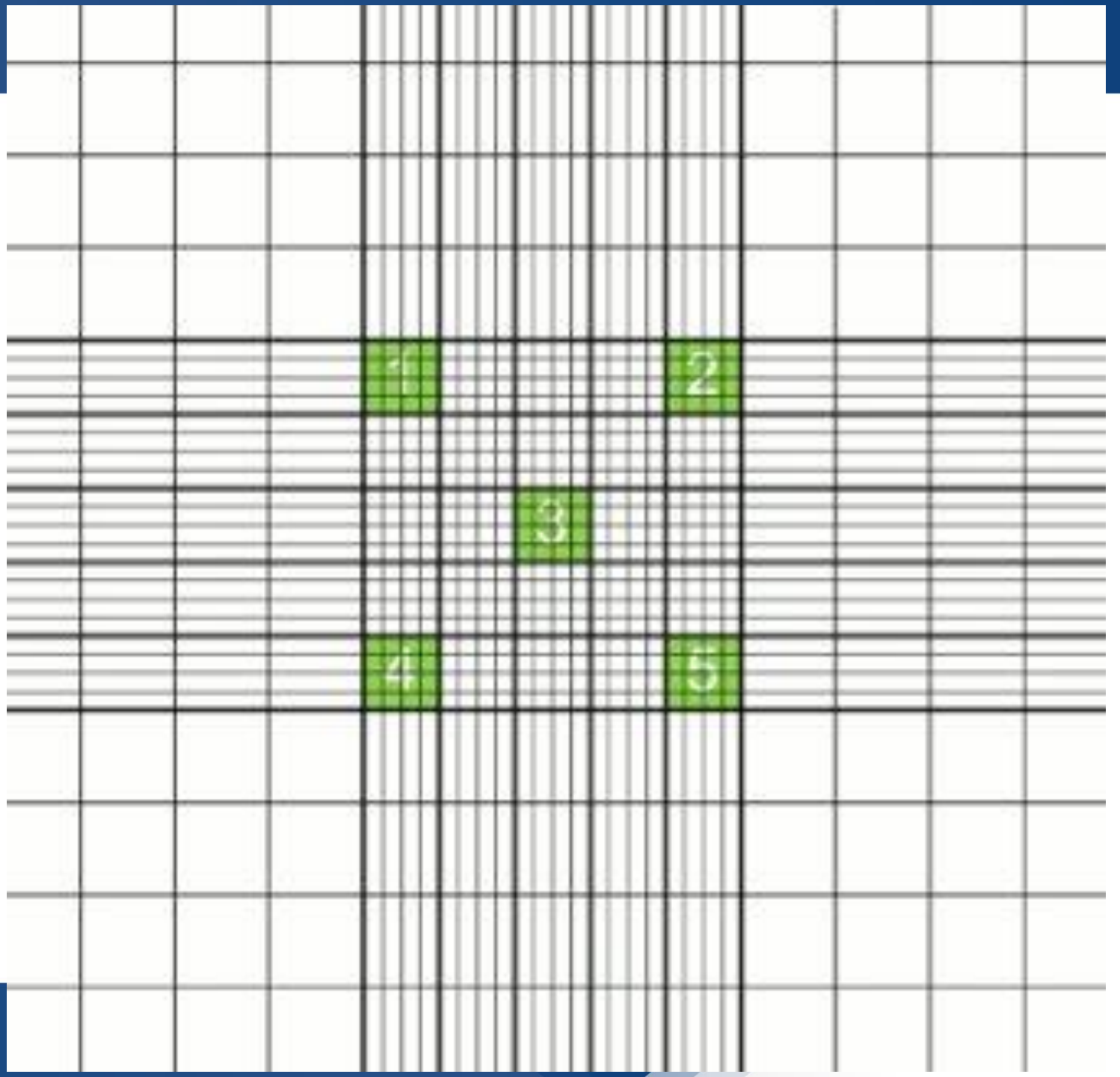


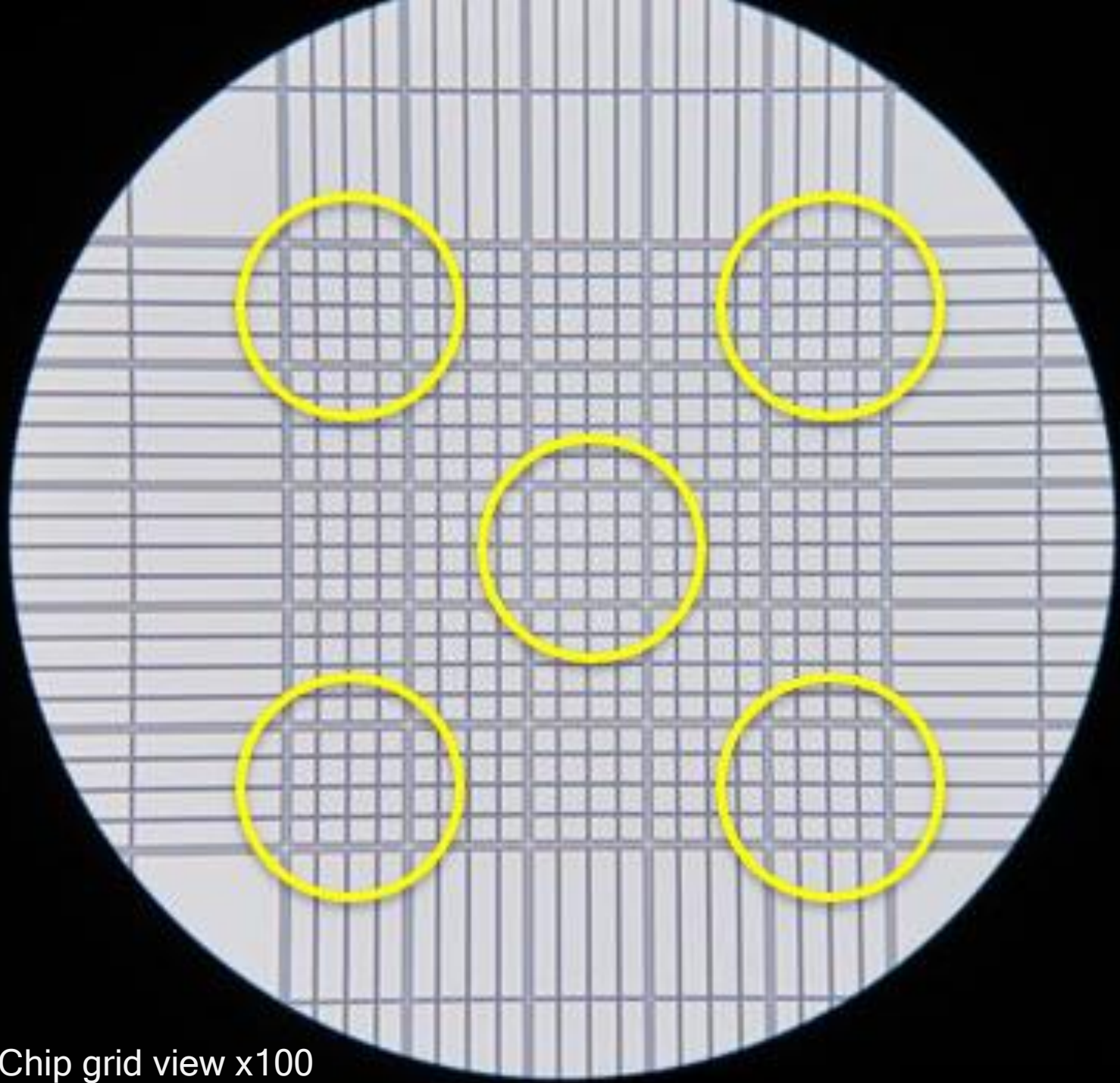




Using the x10 objective lens, view the 25 squares of the counting grid clearly.

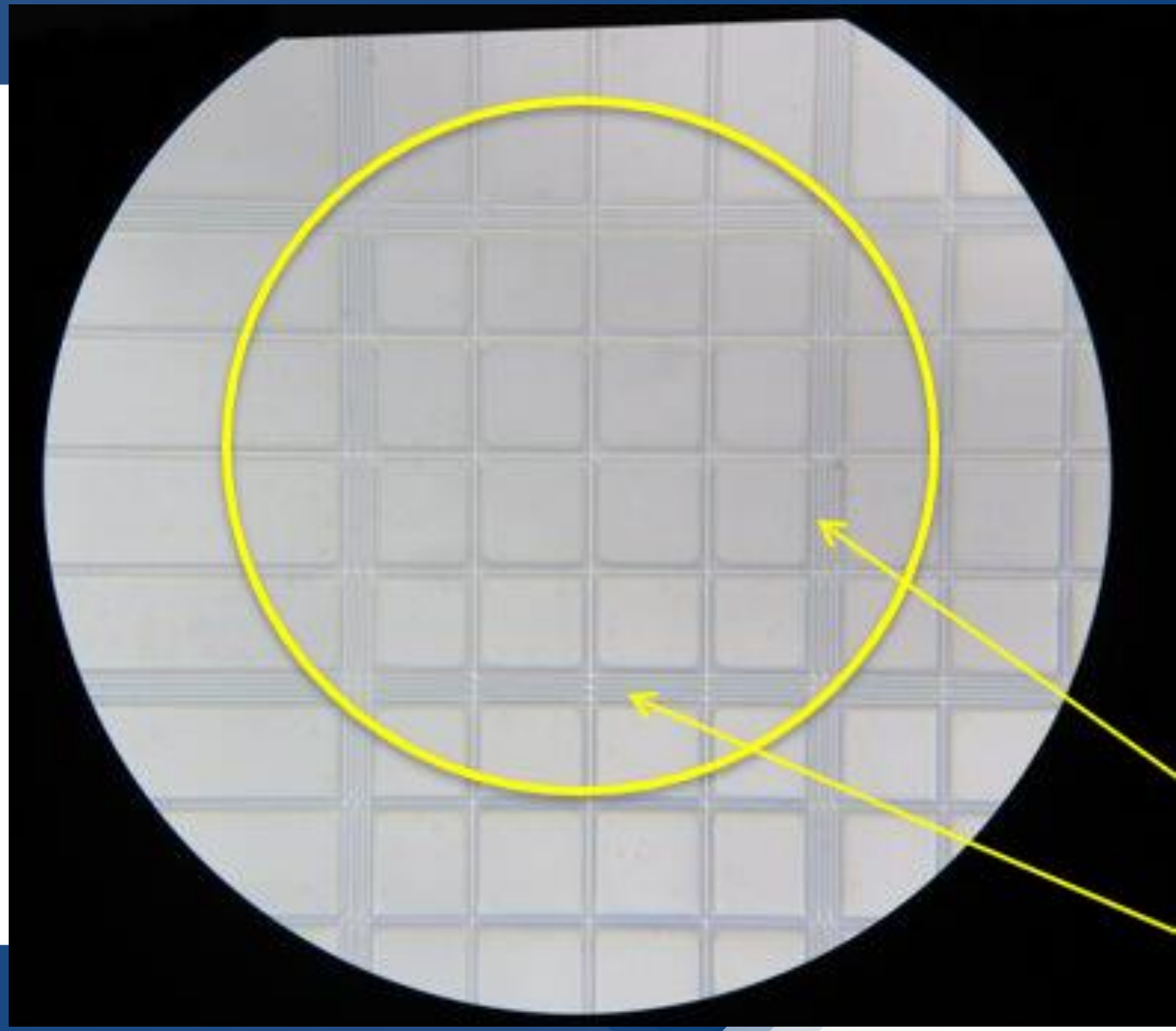
We will sample the 5 squares shown in green in the image below.

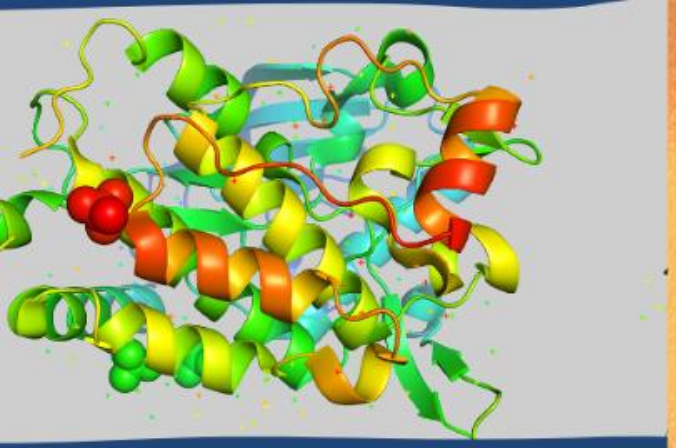




C-Chip grid view x100

Using the x40 objective lens, view the top left-hand box (made up of 16 smaller squares).





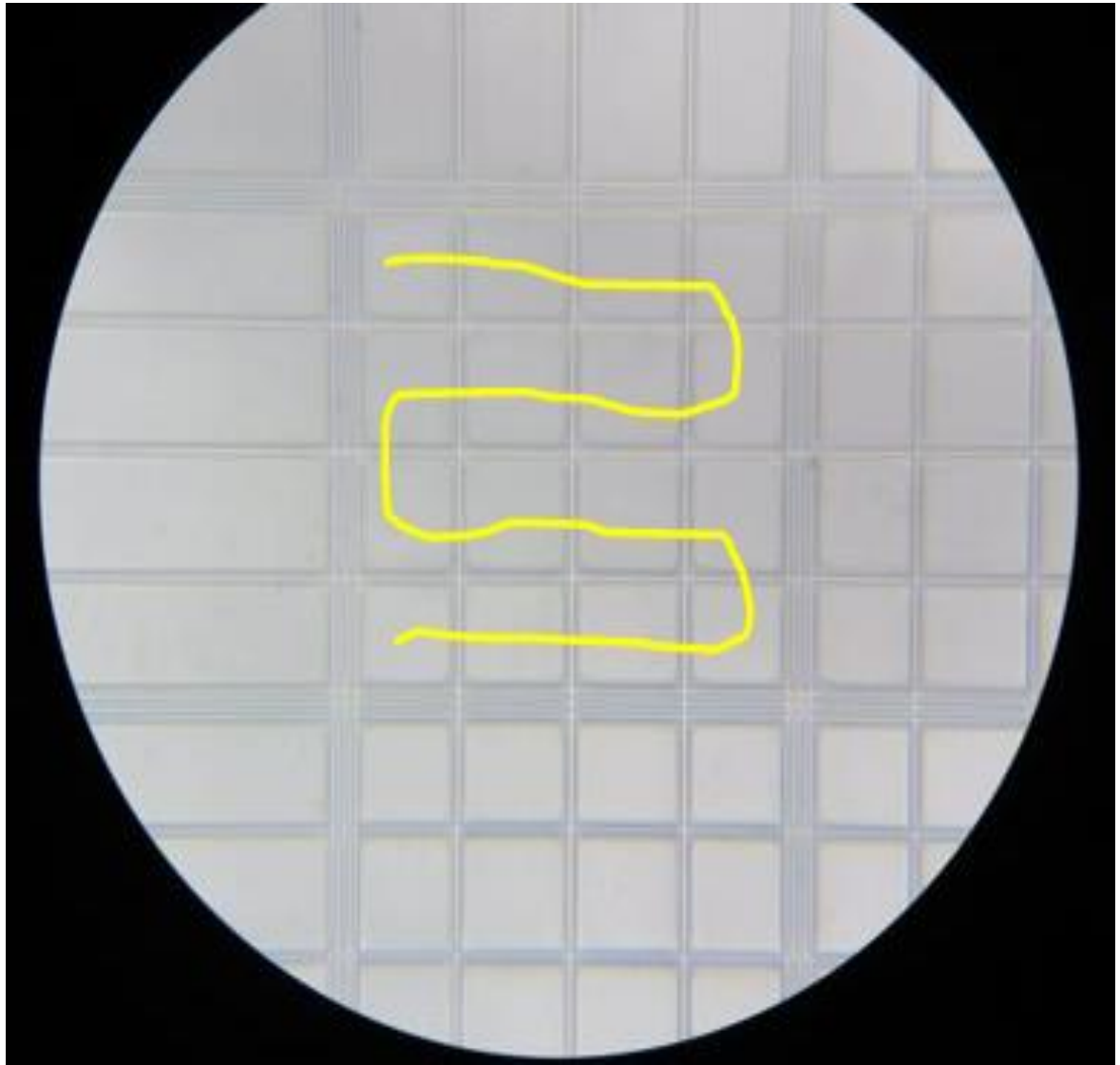
TIME TO COUNT SOME YEAST!

In your pairs:

- Person 1: Load the untreated *S. cerevisiae*
- Person 2: Load the treated culture.

Count systematically

Use this basic rule to ensure consistency in your recorded measurements.



What should be counted?

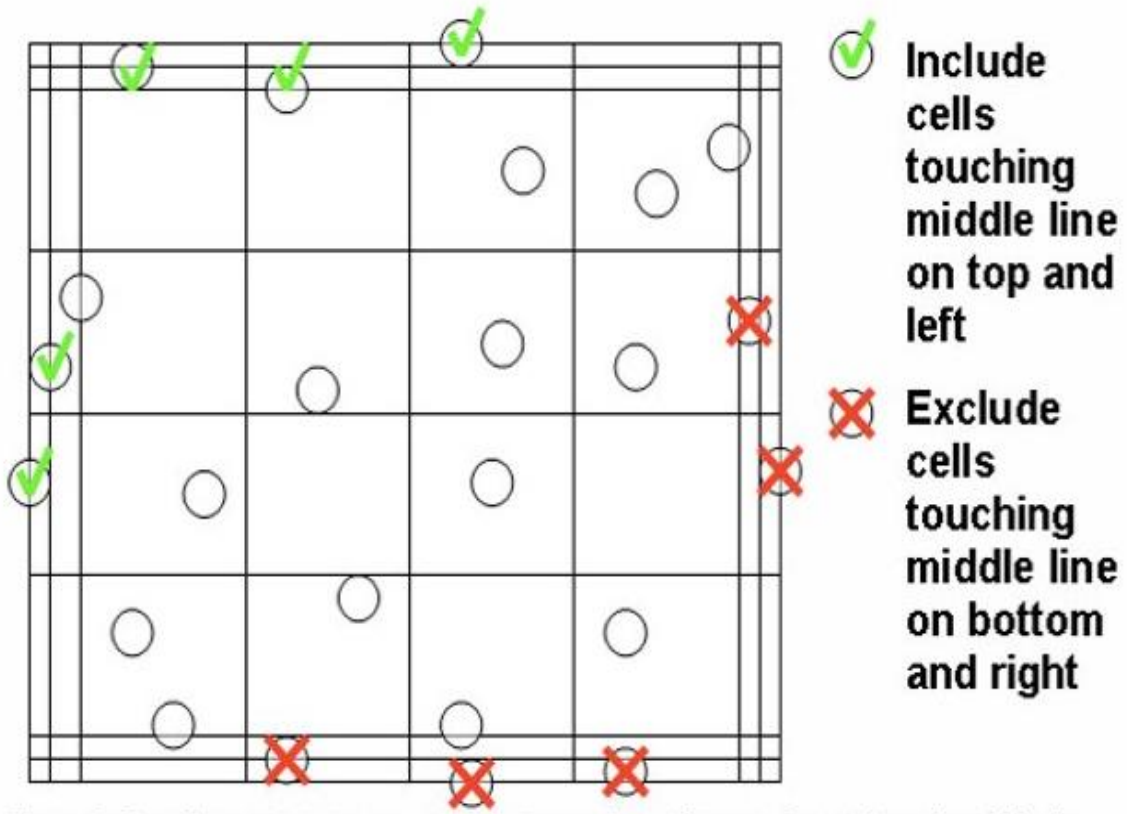
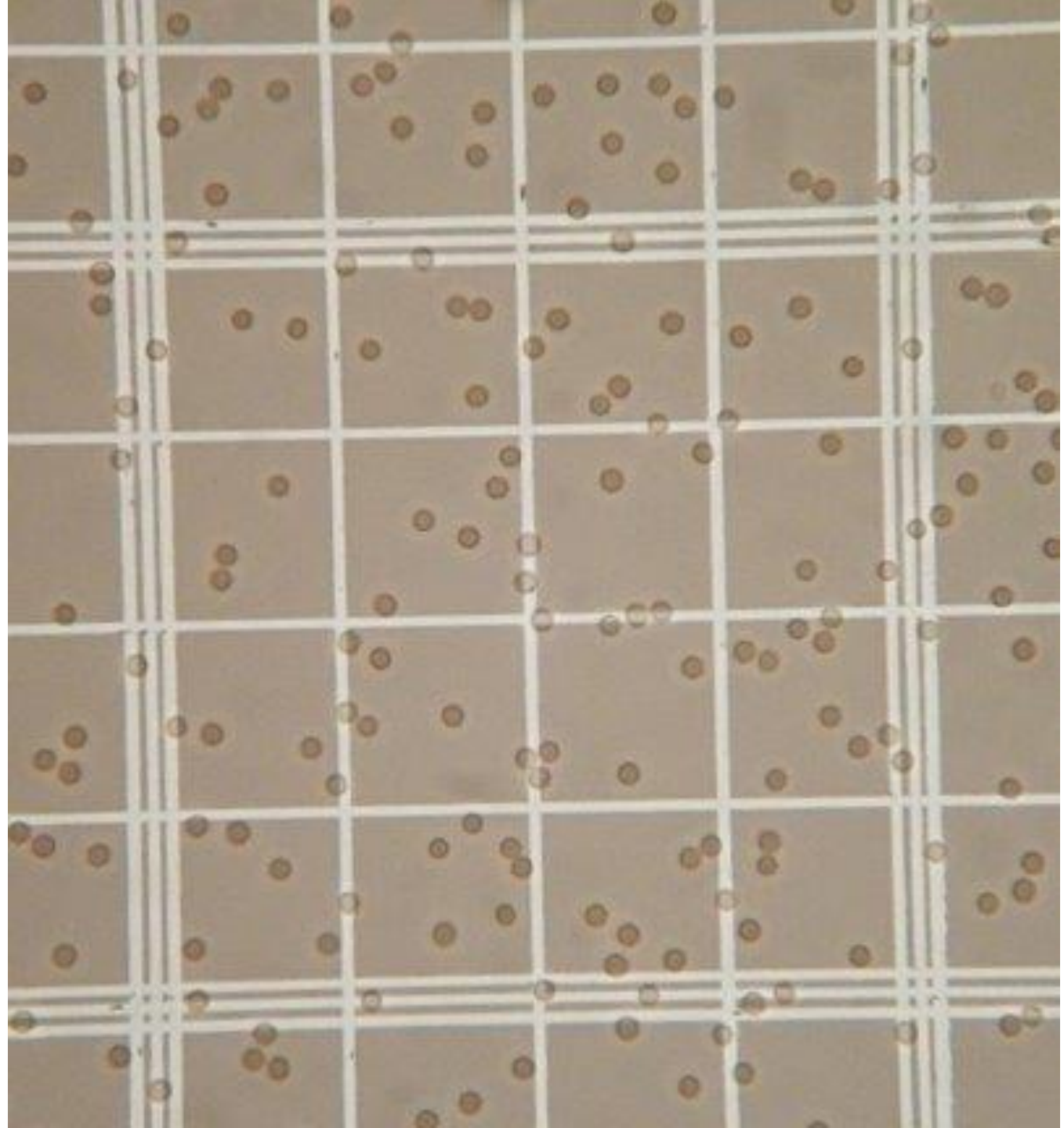


Figure 2. Counting system to ensure accuracy and consistency. Count the cells within the large square and those crossing the edge on two out of the four sides.



Treatment	Number of cells present					No. of cells / 0.02 mm^3	No. of cells / 1mm^3	No. of cells / 1cm^3
	Box 1	Box 2	Box 3	Box 4	Box 5			
None								
Tea tree oil								

Calculations

No. of cells in 5 boxes = 28

Vol. of haemocytometer = $1\,mm \times 1\,mm \times 0.1\,mm$

$= 0.1\,mm^3$ (25 boxes)

$= 0.02\,mm^3$ (5 boxes)

28 cells in $0.02\,mm^3$] x 50

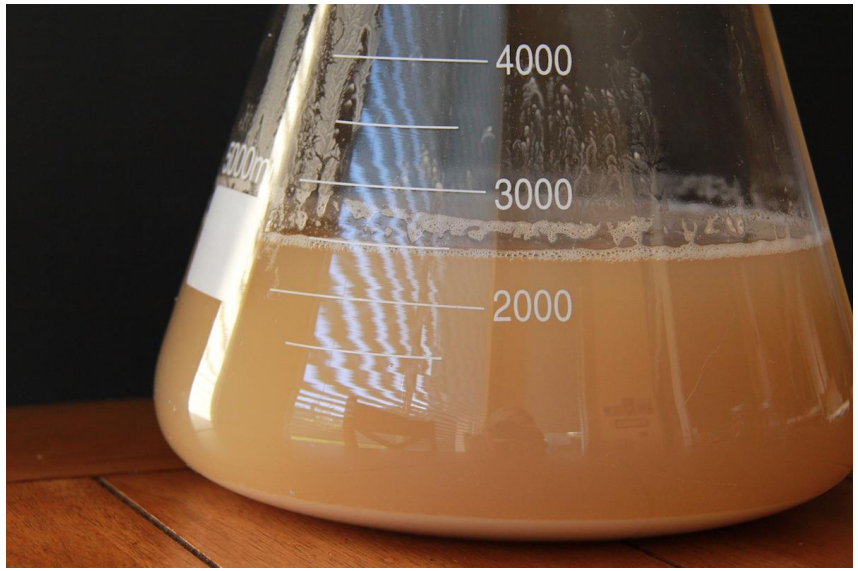
1400 cells in $1\,mm^3$

1400000 cells in $1\,cm^3$] x 1000

$= 1.4 \times 10^6$ cells / cm^3

Alternative approach

Instead of making a direct count of yeast cells using a haemocytometer, a ***colorimeter*** could be used to measure the absorbance of each of cultures.



The Influence of Tea Tree Oil (*Melaleuca alternifolia*) on Fluconazole Activity against Fluconazole-Resistant *Candida albicans* Strains

Anna Mertas, Aleksandra Garbusińska, Ewelina Szliszka, Andrzej Jureczko, Magdalena Kowalska, and Wojciech Król *

Antifungal Effect of Lavender Essential Oil (*Lavandula angustifolia*) and Clotrimazole on *Candida albicans*: An *In Vitro* Study

Fereshteh Behmanesh, [1] Hajar Pasha, [2,*] Ali Asghar Sefidgar, [3] Mohsen Taghizadeh, [4] Ali Akbar Moghadamnia, [5] Hajar Adib Rad, [2] and Leyla Shirkhani [6]

Effect of Essential Oils on Pathogenic Bacteria

Filomena Nazzaro,[1,*] Florinda Fratianni,[1] Laura De Martino,[2] Raffaele Coppola,[1] and Vincenzo De Feo[2]

Abstract

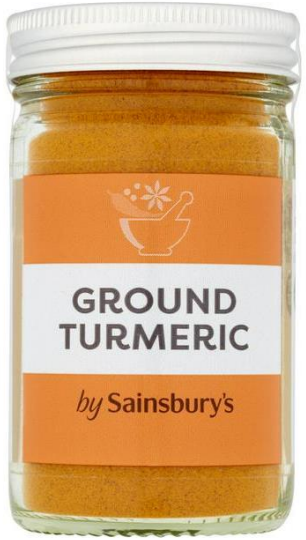
Go to: ▸

The increasing resistance of microorganisms to conventional chemicals and drugs is a serious and evident worldwide problem that has prompted research into the identification of new biocides with

Curcumin Inhibits Growth of *Saccharomyces cerevisiae* through Iron Chelation

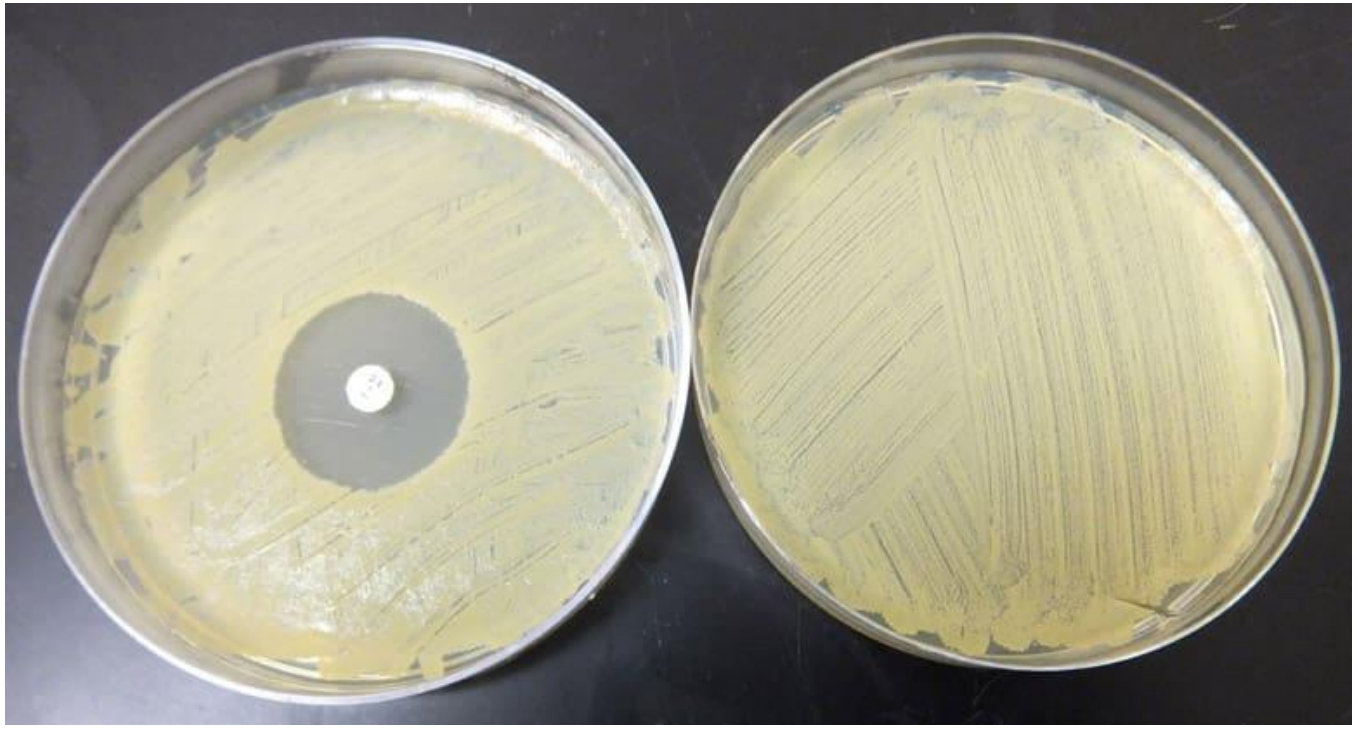
Steven Minear,[1,‡†] Allyson F. O'Donnell,[1,§†] Anna Ballew,[1,¶] Guri Giaever,[2,‖] Corey Nislow,[2,#] Tim Stearns,[1,2] and Martha S. Cyert[1,*]

Active component of turmeric



Much safer than using antibiotics and provides a sound "justification" for looking at alternatives for human health.

Microbiology – confounding variables



SQA has made comments about the challenges of using the "disc diffusion method". If this was the **same** chemical applied to each disc, this would be ok. However, if **different** chemicals are being **compared**, the disc diffusion method would not be seen as an **appropriate method** and too many confounding variables.