

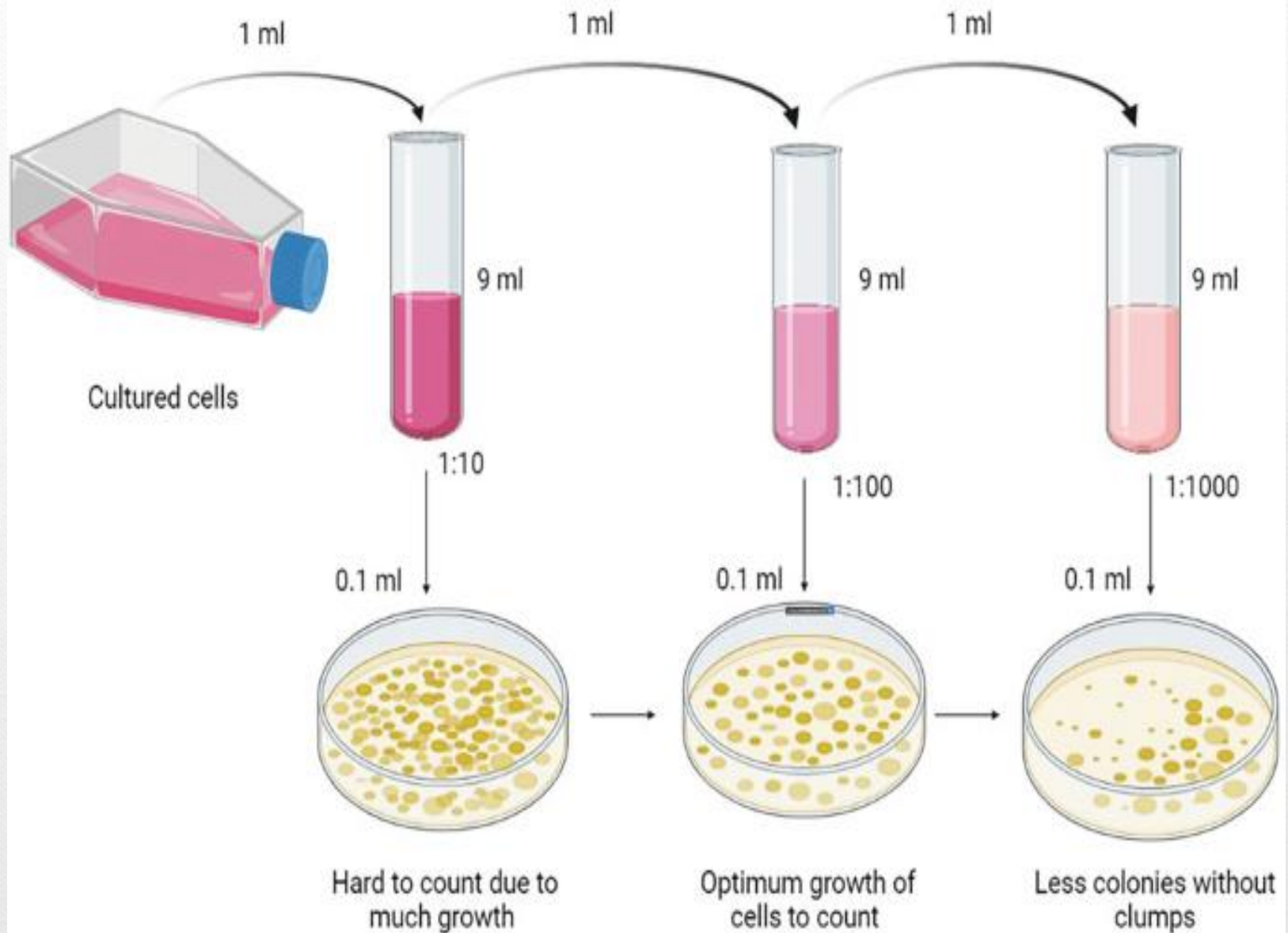
Cell Passage and Quantitation Methods

تمرير الخلايا وطرق حسابها

Lec2

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Introduction to Cell Culture

- Definition: Growth of cells in an artificial environment.
- Primary vs continuous cell lines.

The reasons for Cell Passage

أسباب تمرير الخلايا

- To prevent Merge cells
- Maintain viability.
- Provide nutrients for growth.
- Prevent cell crowding and regenerate green cells.

Trypsinization Process

عملية التريپسين

- Trypsin detaches adherent cells.
- Steps: Wash → Add Trypsin → Incubate → Neutralize → Centrifuge → Resuspend.

Trypsinization

Rinse Monolayer Culture



Add Trypsin-EDTA



Incubate



Stop Trypsinization



Spin Cells



Add Fresh Medium



Count cells



Seed new flask



Incubate at 37°C

Cell Quantitation Methods

طرق قياس عدد الخلايا

- 1. Manual Counting (Hemocytometer)
- 2. Electronic Counting (Coulter Counter)
- 3. Flow Cytometry
- 4. Spectrophotometry

Manual Counting (Hemocytometer Counting)

1- العد اليدوي

- Glass slide with grid for manual cell counting.
- **Trypan Blue test** distinguishes live and dead cells.
- Formula: $(\text{Count} \times \text{Dilution} \times 10^4) / \text{Squares Counted}$.



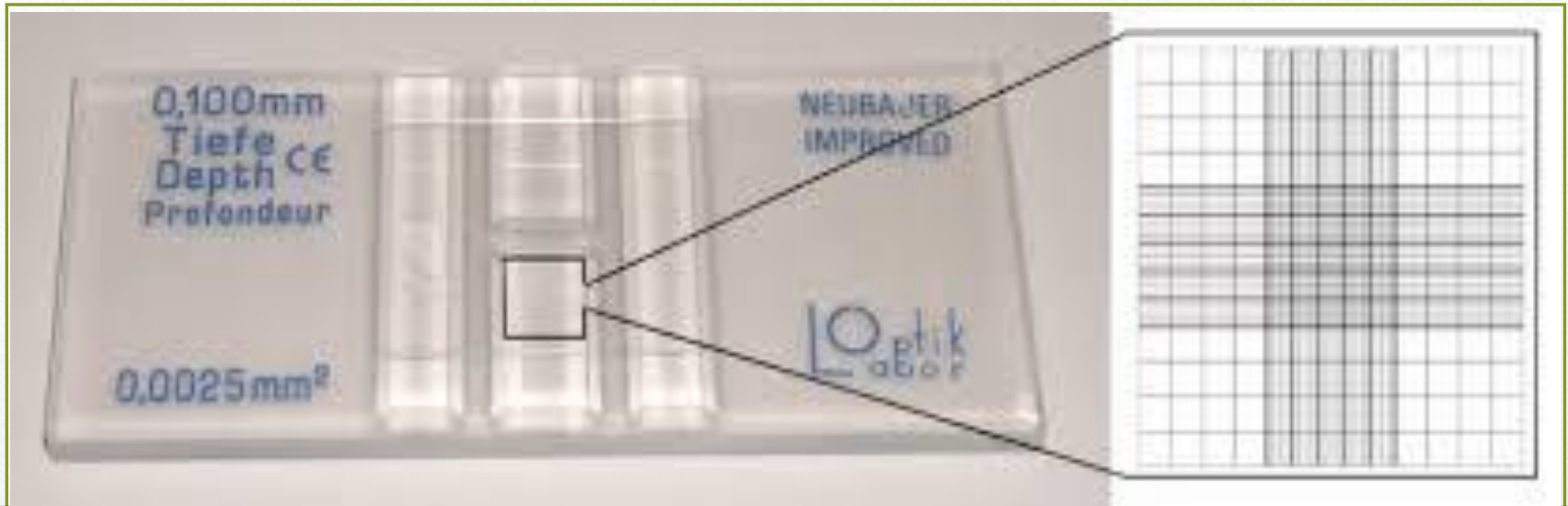
نشاط

ما هو الموت المبرمج للخلايا

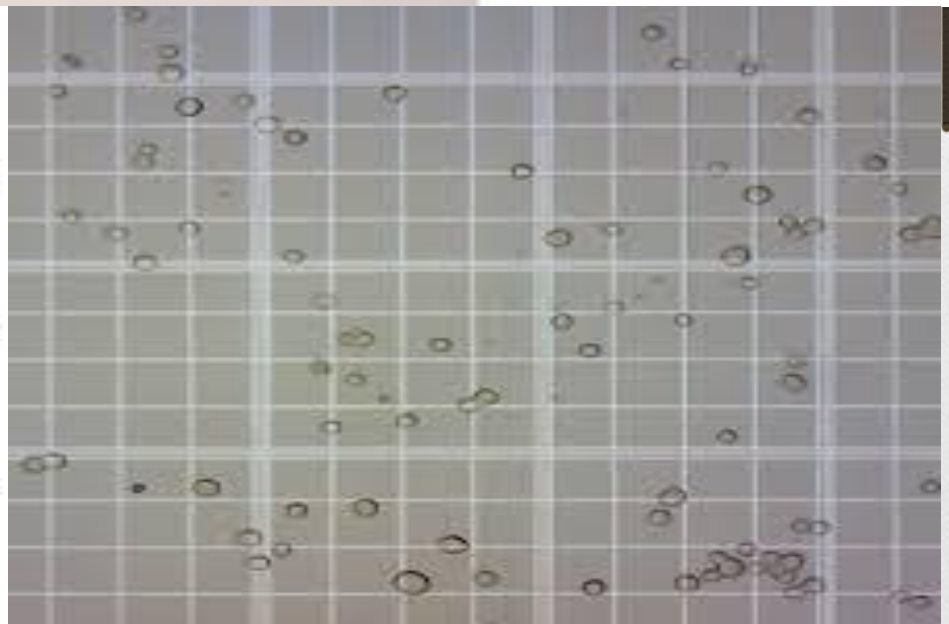
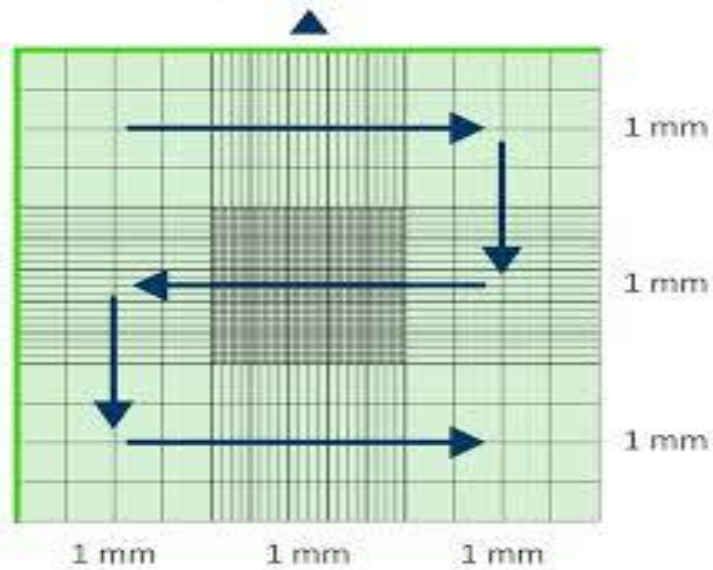


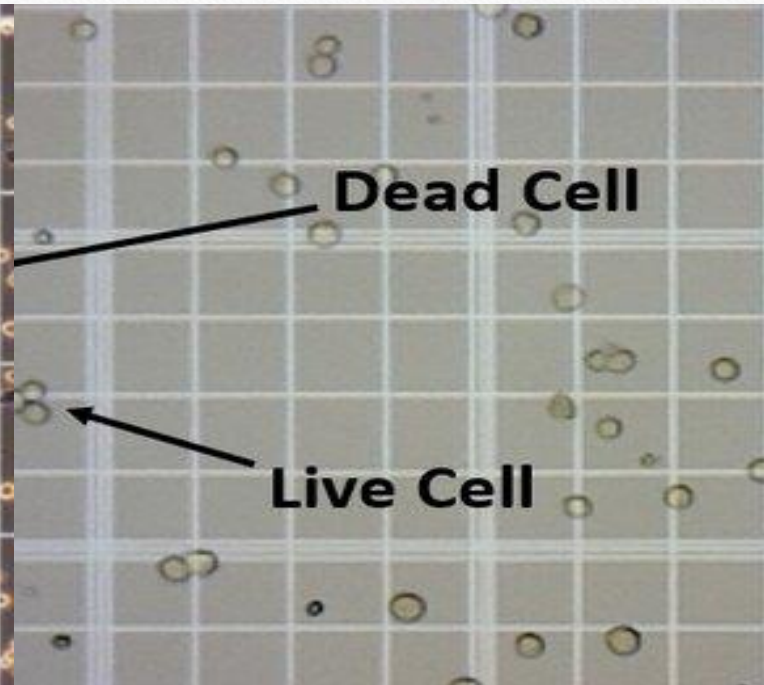
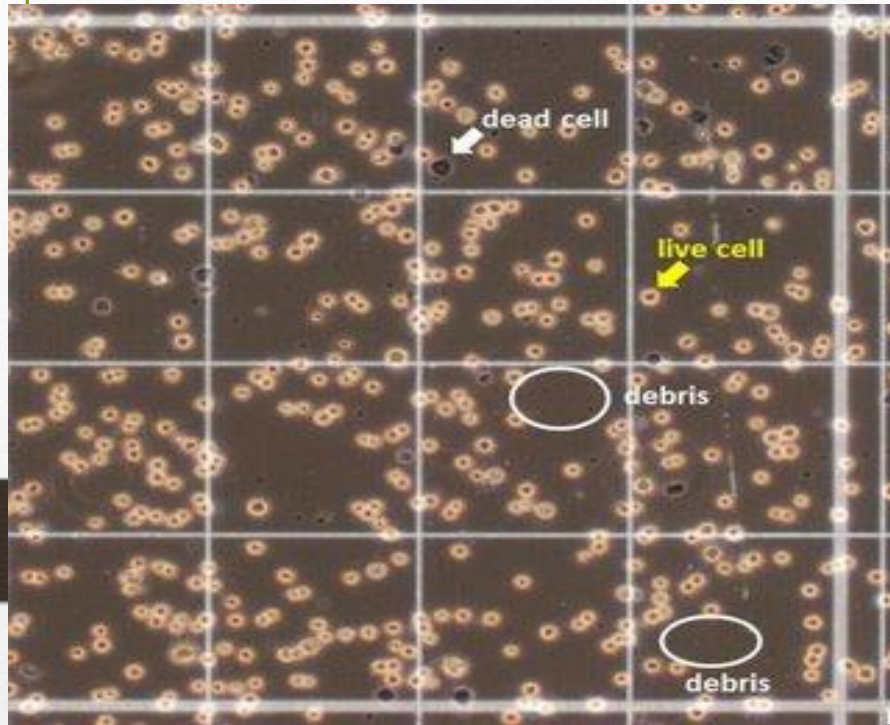
Hemocytometer Counting Procedure

- Glass slide with a grid for manual counting.
- - Procedure:
 1. Mix cells with Trypan Blue
 2. Load into chamber
 3. Count under microscope
 4. Apply calculation formula
- - Pros: Simple, cheap, viability check
- - Cons: Time-consuming, user error



The absolute count





Electronic Counting

2 العد الإلكتروني

- Cells pass through a small aperture; resistance change counted.
- **Advantages**: fast, high Production.
- **disadvantages**: expensive, waste may be counted as cells.



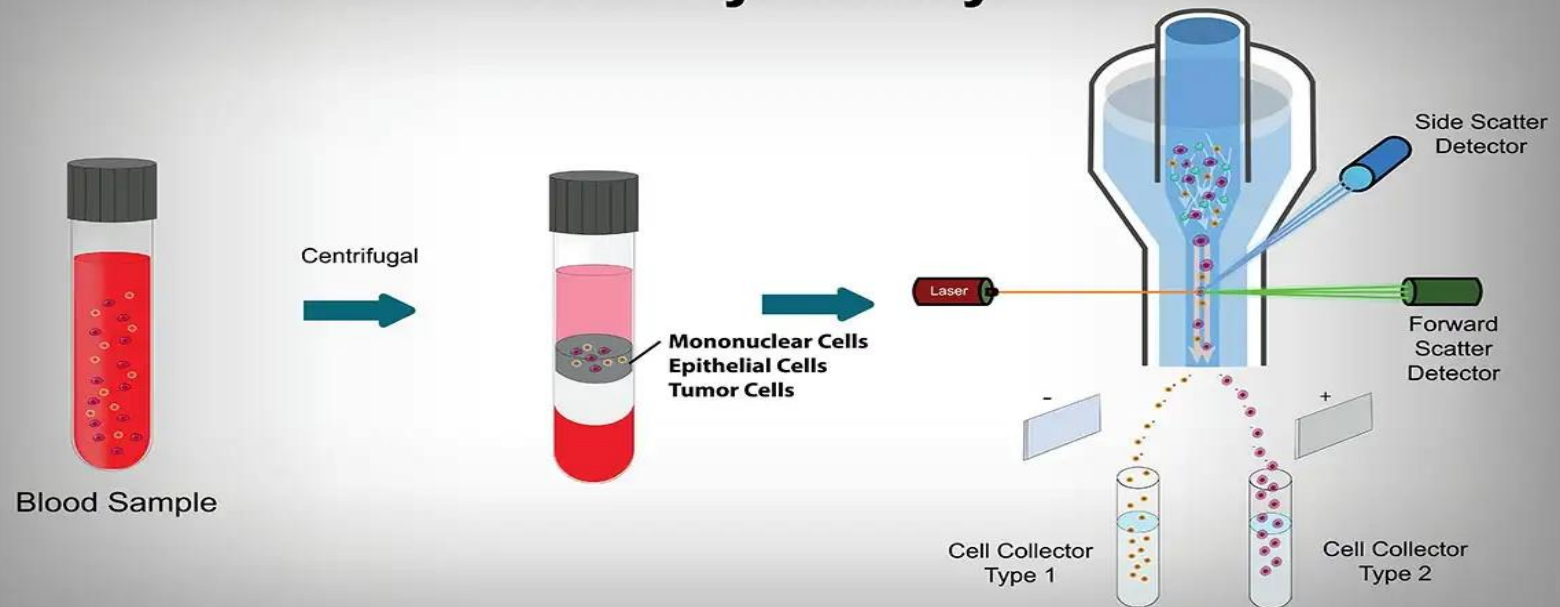
Flow Cytometry

3 القياس بالتدفق الخلوي

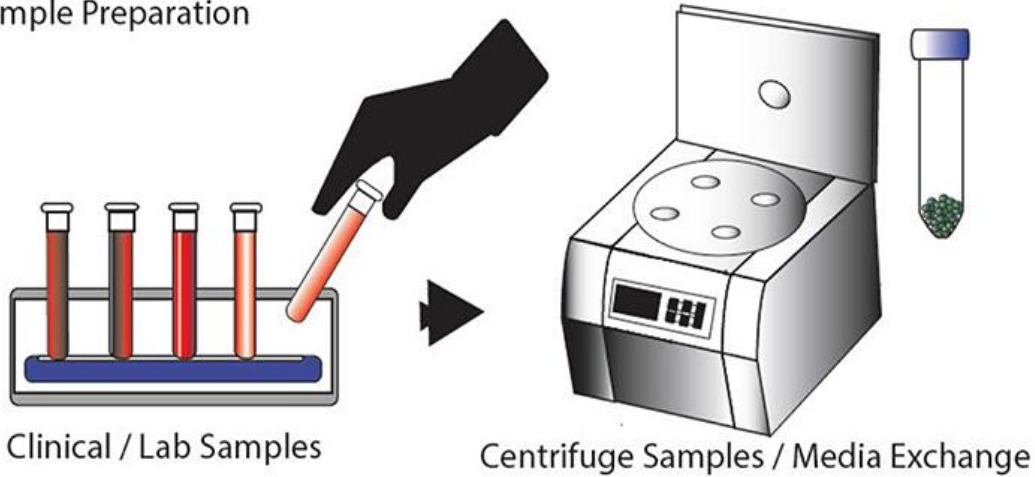
- **Measures cell size, granularity, fluorescence.**
- **Used for viability and apoptosis studies.**



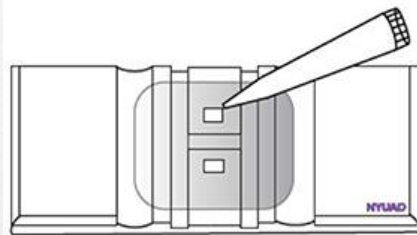
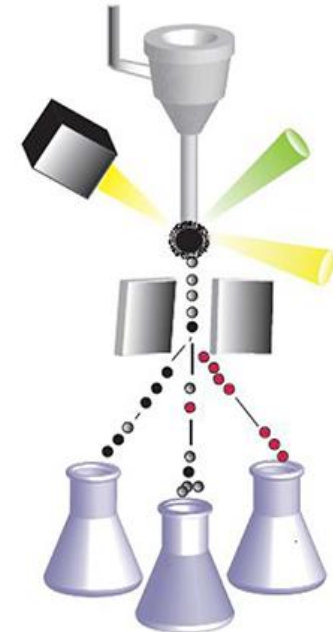
Flow Cytometry



Sample Preparation



Flow Cytometer



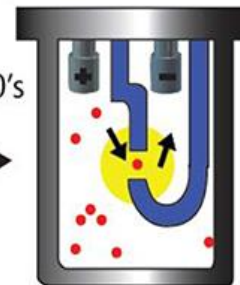
Hemocytometer

19th
Century



Plating

1950's

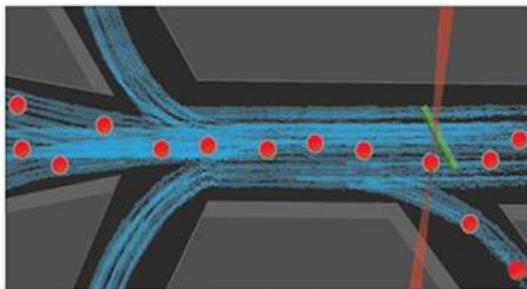


Impedance counter

1960's



1990's



Microfluidic Cytometer

2000's

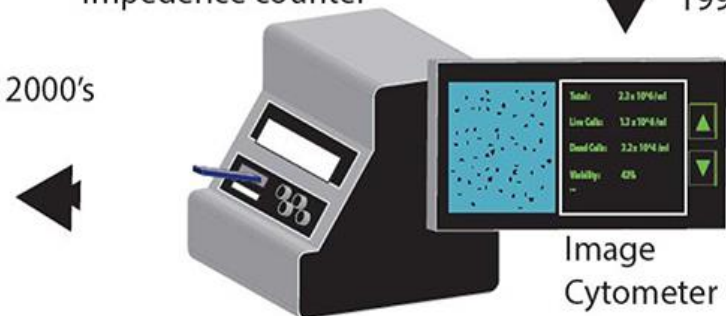
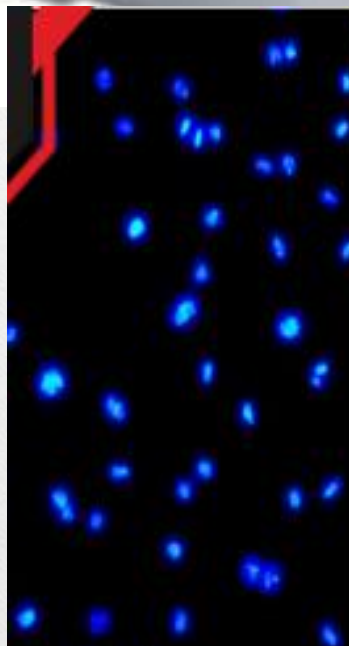
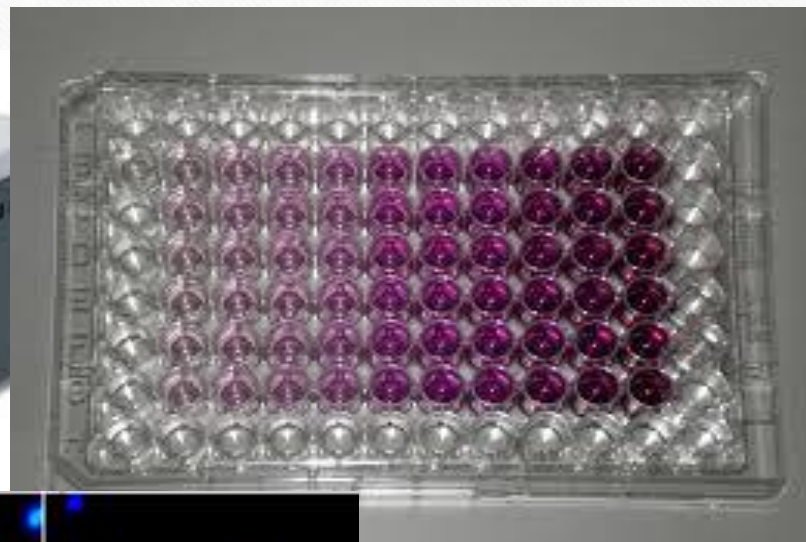


Image
Cytometer

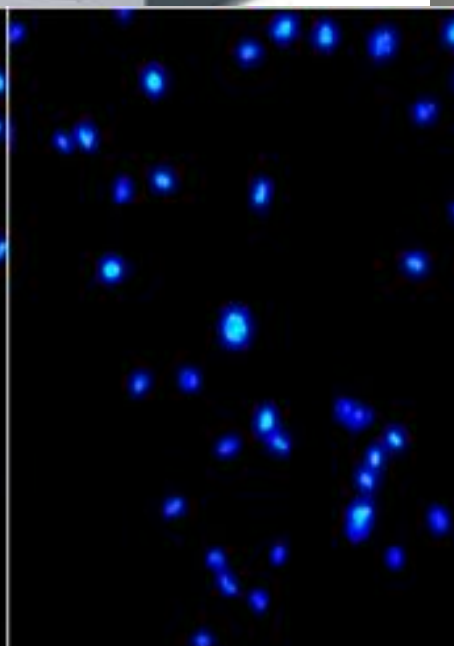
Spectrophotometric Quantitation

4 القياس الطيفي

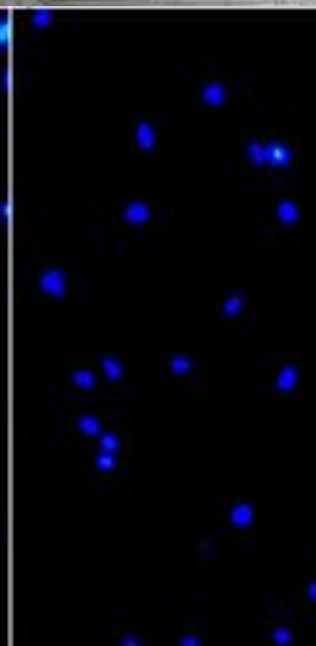
- Based on absorbance (OD) correlation with cell number.
- Common assays: MTT, Crystal Violet.
- Flow Cytometry: counts + analyzes thousands/sec, fluorescence markers.
- Spectrophotometry (OD): measures turbidity, mainly microbes.
- Automated Imaging: real-time cell monitoring, confluency, morphology.



24h



48h



72h

Biosafety and Ethics

السلامة والأخلاقيات

- Use PPE.
- Proper disposal of waste.
- Maintain integrity and accuracy in lab work.