In order to study which phase of the **cell (tumour) process** is influenced by the molecule under examination, experimental models are used.

They are distinguished as:

1. **monovariable models**

they are performed **only in vitro** since a single phase of the process is evaluated

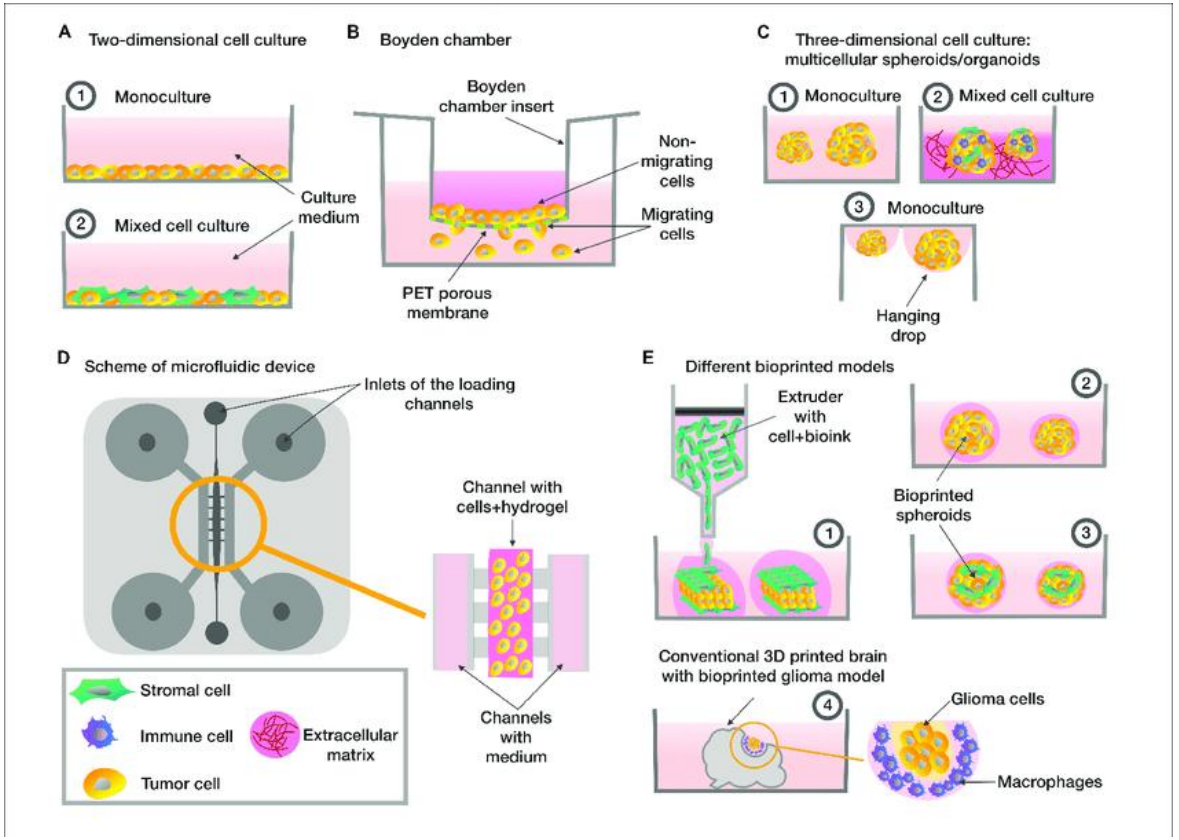
2. **Multivariable models**
they include multiple process steps, they can be **in vitro and in vivo** (using mice or rats.)

in vitro models

- They allow to evaluate:

- Adhesion
- Spreading
- migration
- Invasion

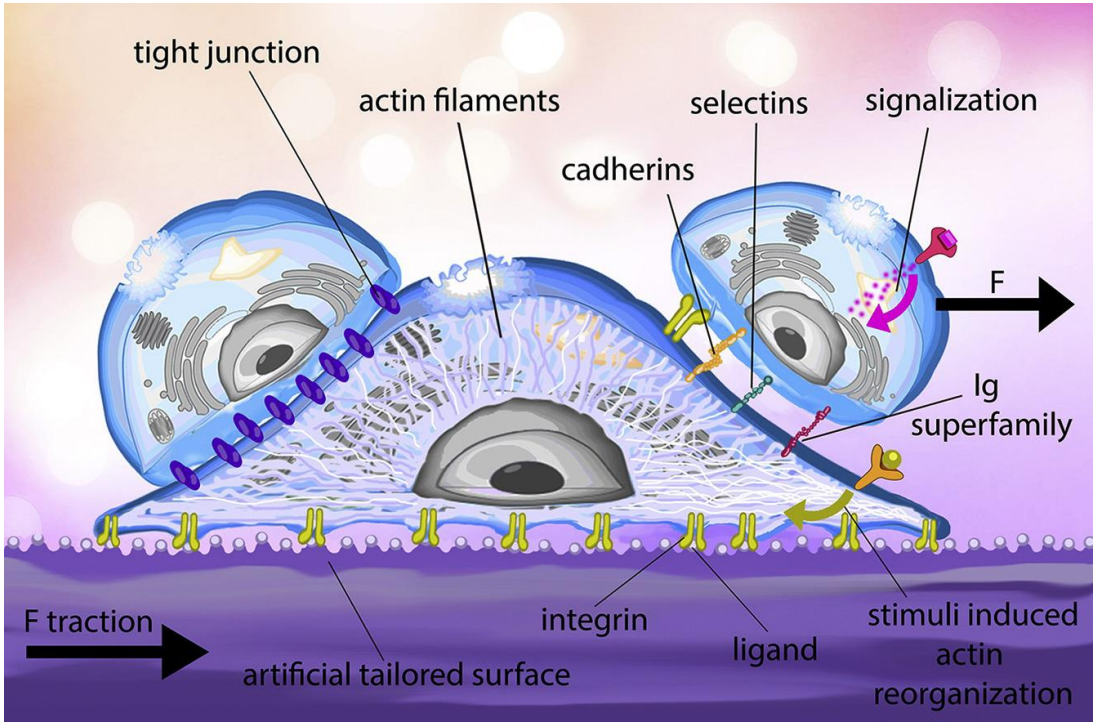
- Proliferation



Cell adhesion

is a **complex process** involved in migration/invasion, embryogenesis, wound healing and tissue remodeling.

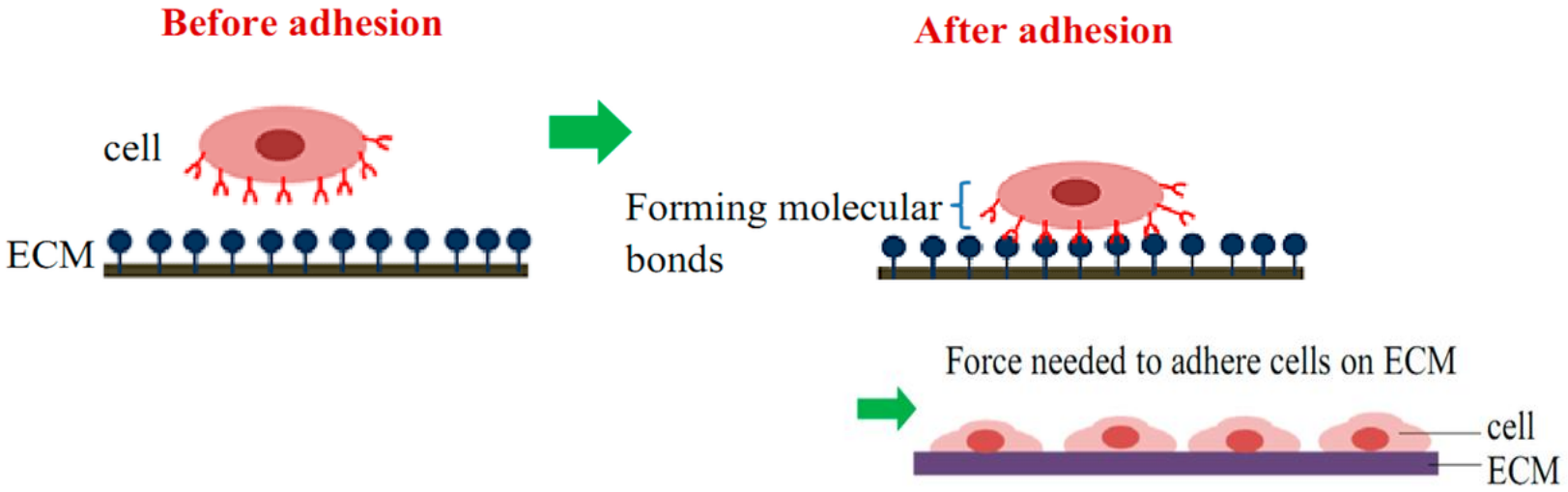
Cells **adhere to the extracellular matrix**, forming complexes with cytoskeleton components that can affect cell motility, differentiation, proliferation, and survival.



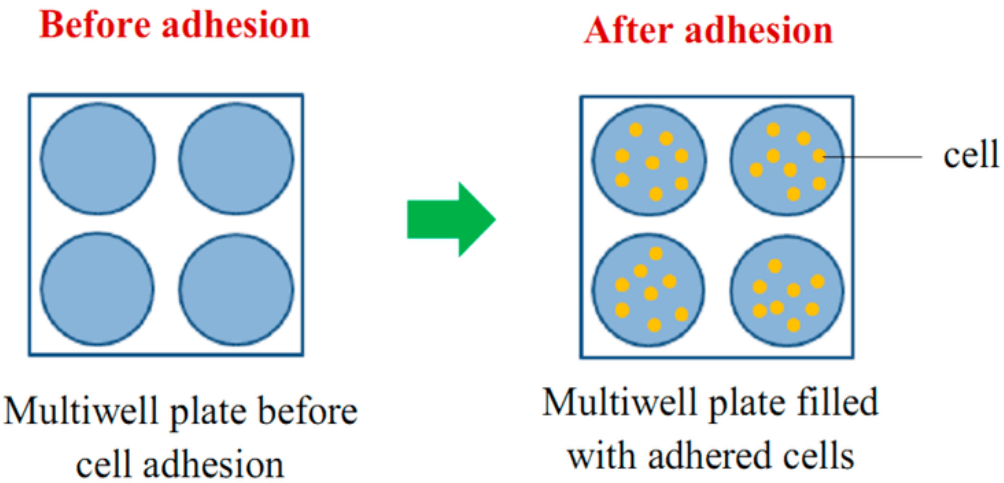
Cell adhesion

- Cells are seeded onto the substrate.

- **Adherent cells** attach, while non-adherent cells are washed away.



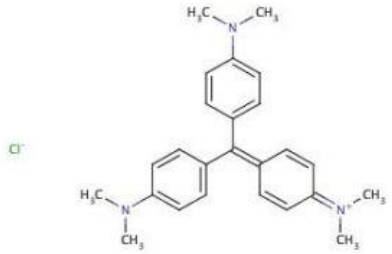
- Adherent cells **are quantified**.

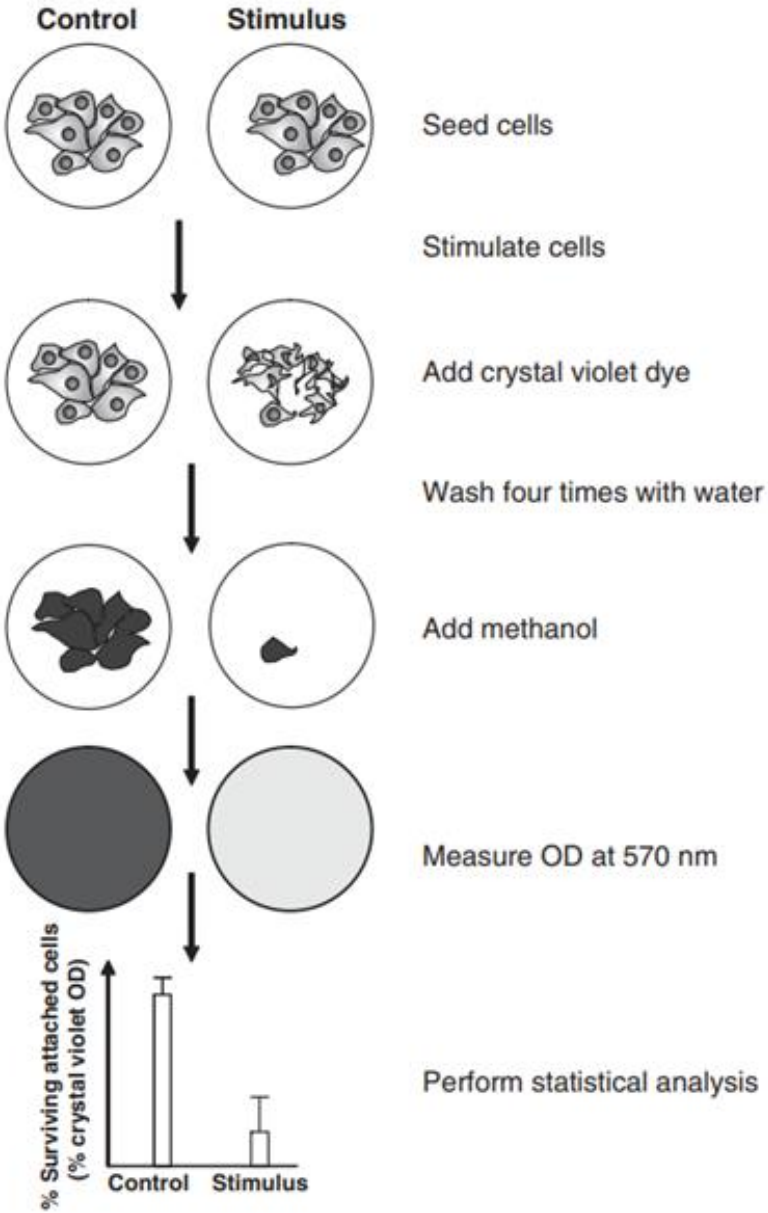


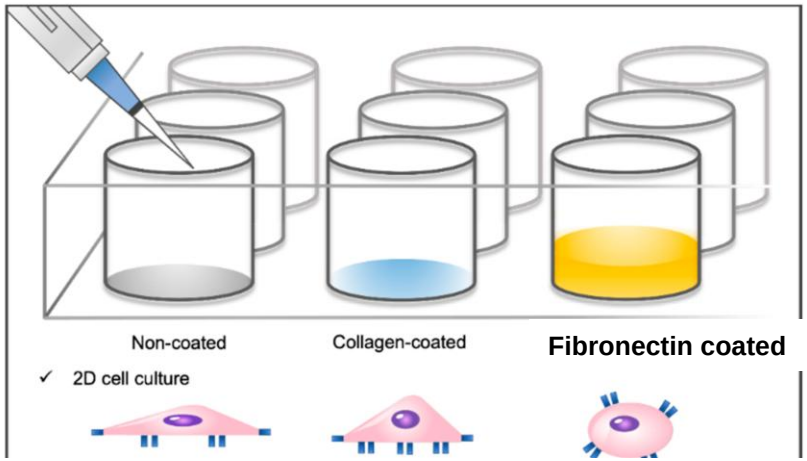
- **Crystal Violet** staining

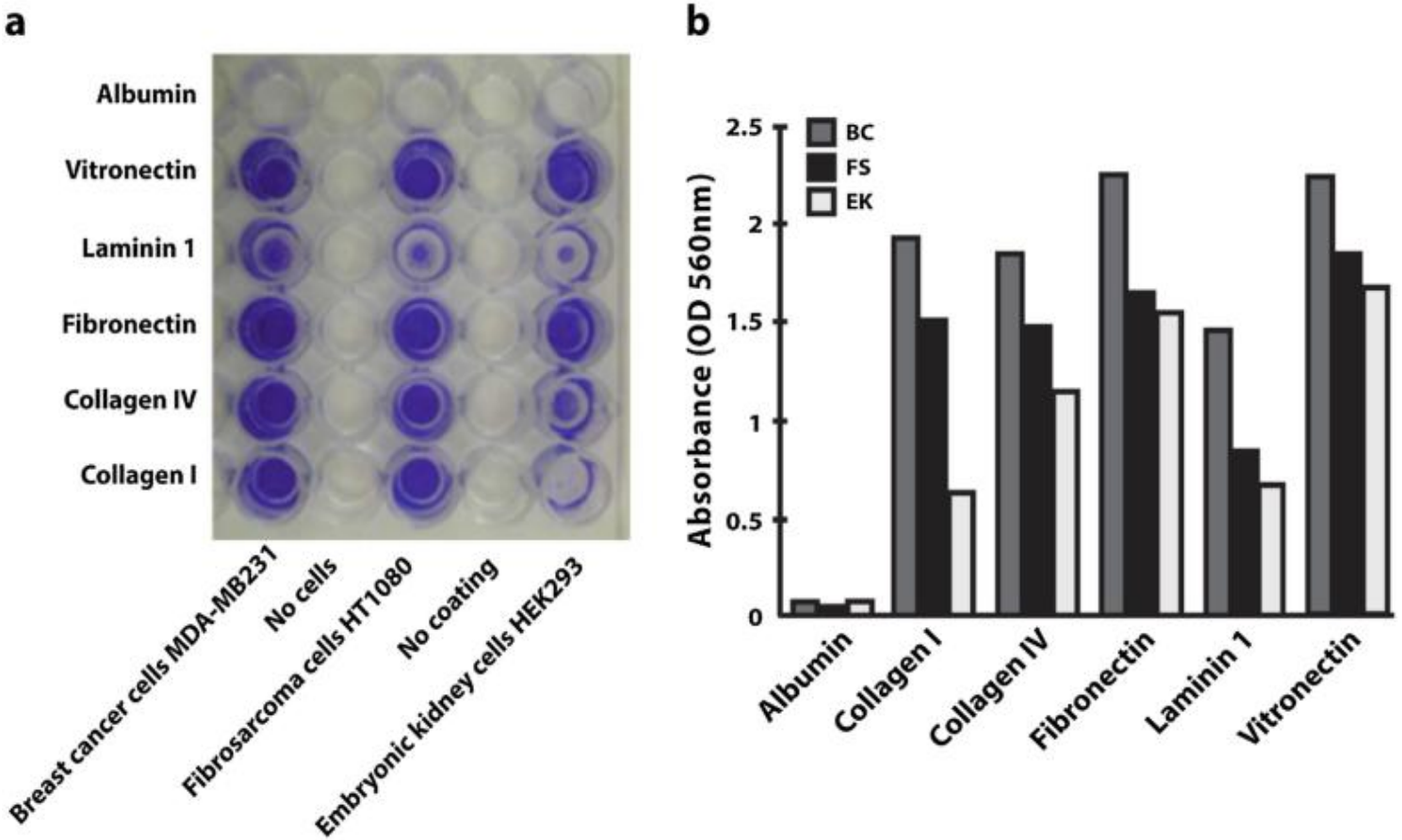
Crystal violet stains **nucleic acids and proteins**

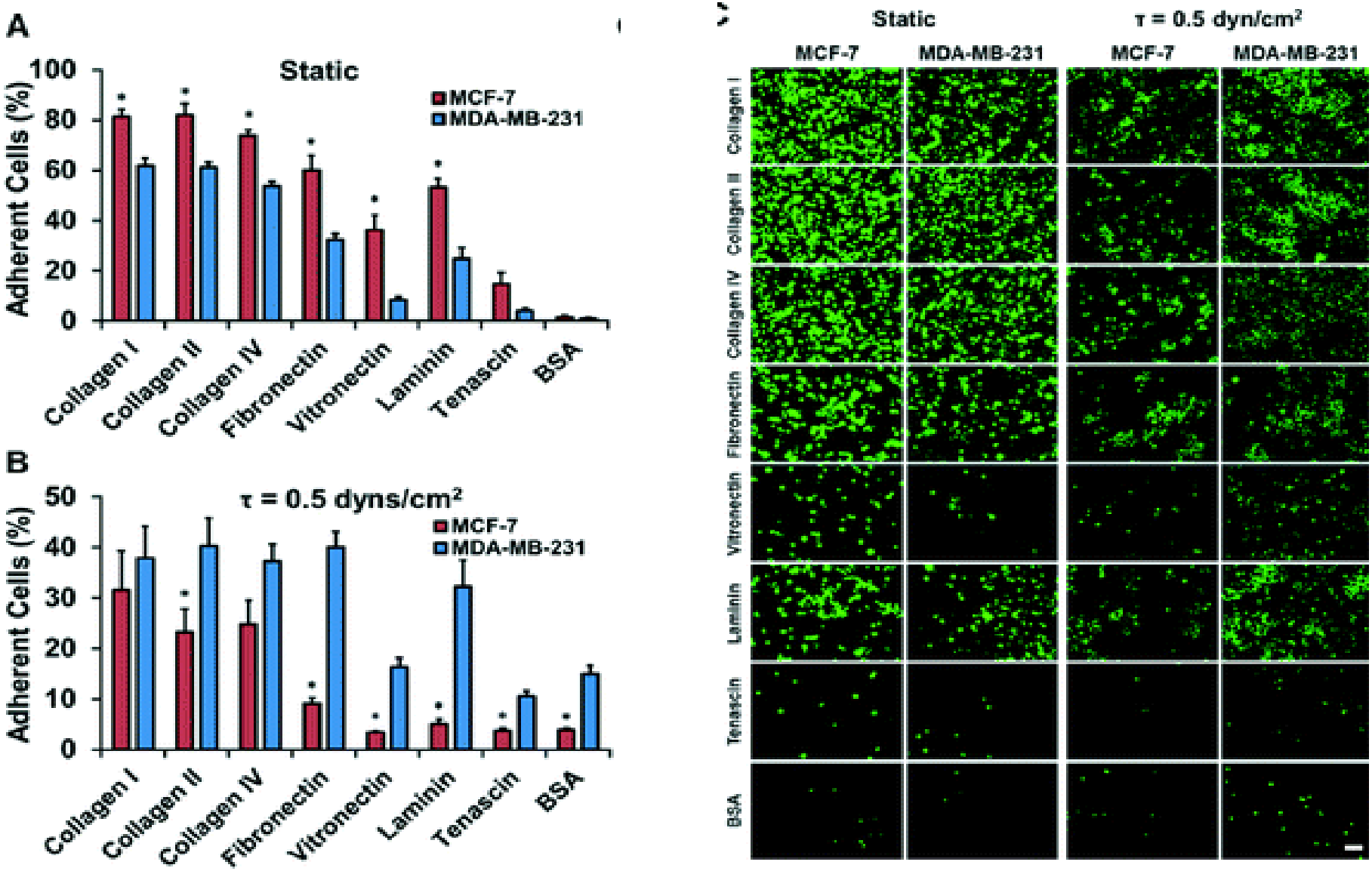
It is used to reflect the **cell number in 96-well plates**.






Non-coated
Collagen-coated
Fibronectin coated
✓ 2D cell culture

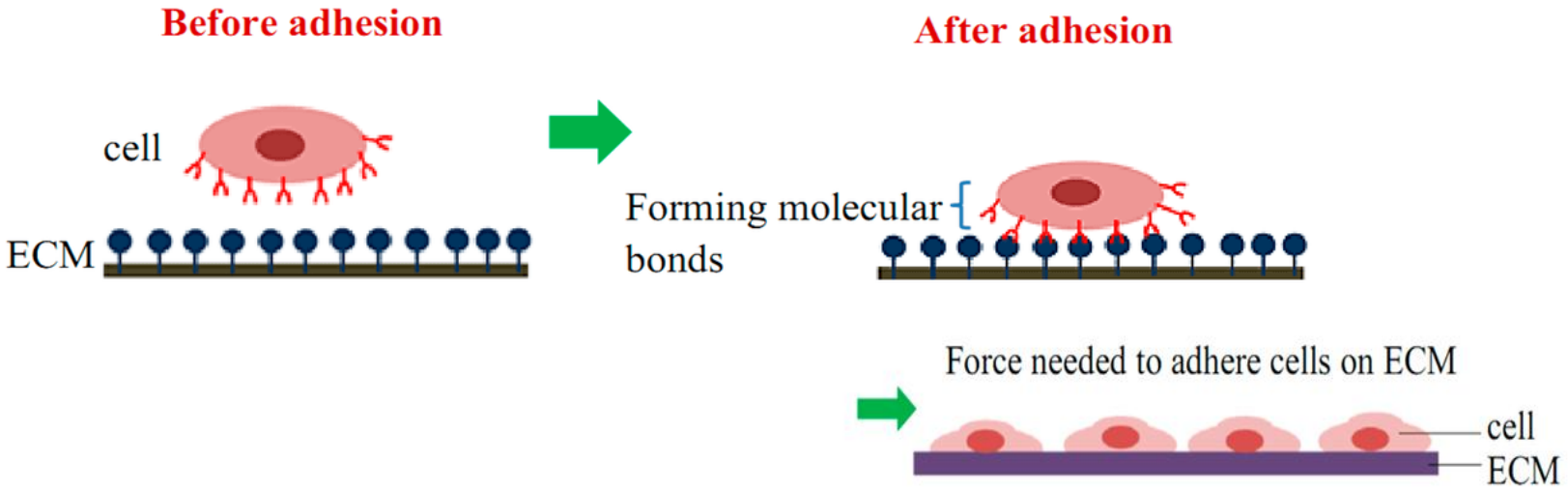

a
Albumin
Vitronectin
Laminin 1
Fibronectin
Collagen IV
Collagen I
Breast cancer cells MDA-MB231
No cells
Fibrosarcoma cells HT1080
No coating
Embryonic kidney cells HEK293
b
Absorbance (OD 560nm)
2.5
2
1.5
1
0.5
0
BC
FS
EK
Albumin
Collagen I
Collagen IV
Fibronectin
Laminin 1
Vitronectin


A
Static
MCF-7
MDA-MB-231
Adherent Cells (%)
100
80
60
40
20
0
Collagen I
Collagen II
Collagen IV
Fibronectin
Vitronectin
Laminin
Tenascin
BSA
B
τ = 0.5 dyns/cm²
MCF-7
MDA-MB-231
Adherent Cells (%)
50
40
30
20
10
0
Collagen I
Collagen II
Collagen IV
Fibronectin
Vitronectin
Laminin
Tenascin
BSA
Static
τ = 0.5 dyn/cm²
MCF-7
MDA-MB-231
MCF-7
MDA-MB-231
Collagen I
Collagen II
Collagen IV
Fibronectin
Vitronectin
Laminin
Tenascin
BSA

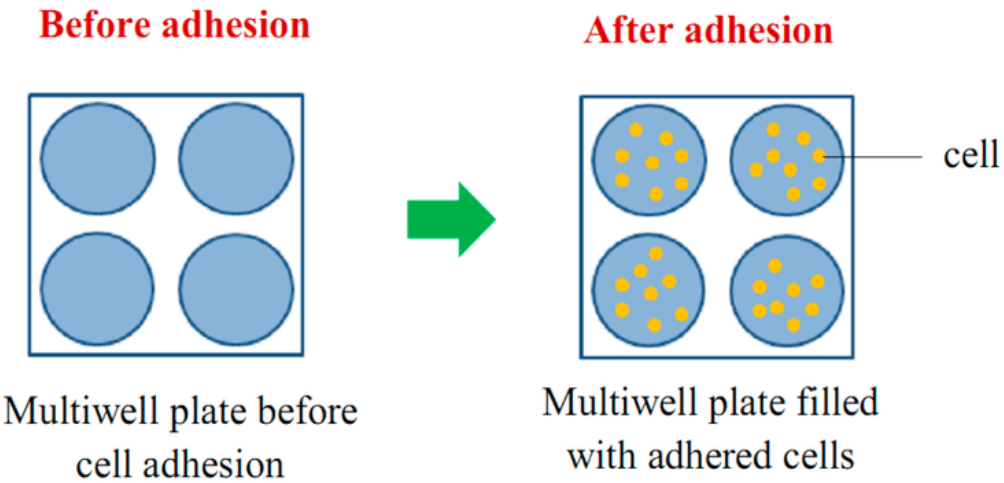
Cell adhesion

- Cells are seeded onto the substrate.

- **Adherent cells** attach, while non-adherent cells are washed away.

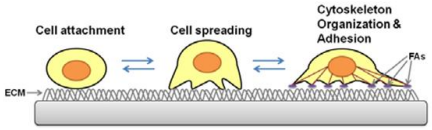


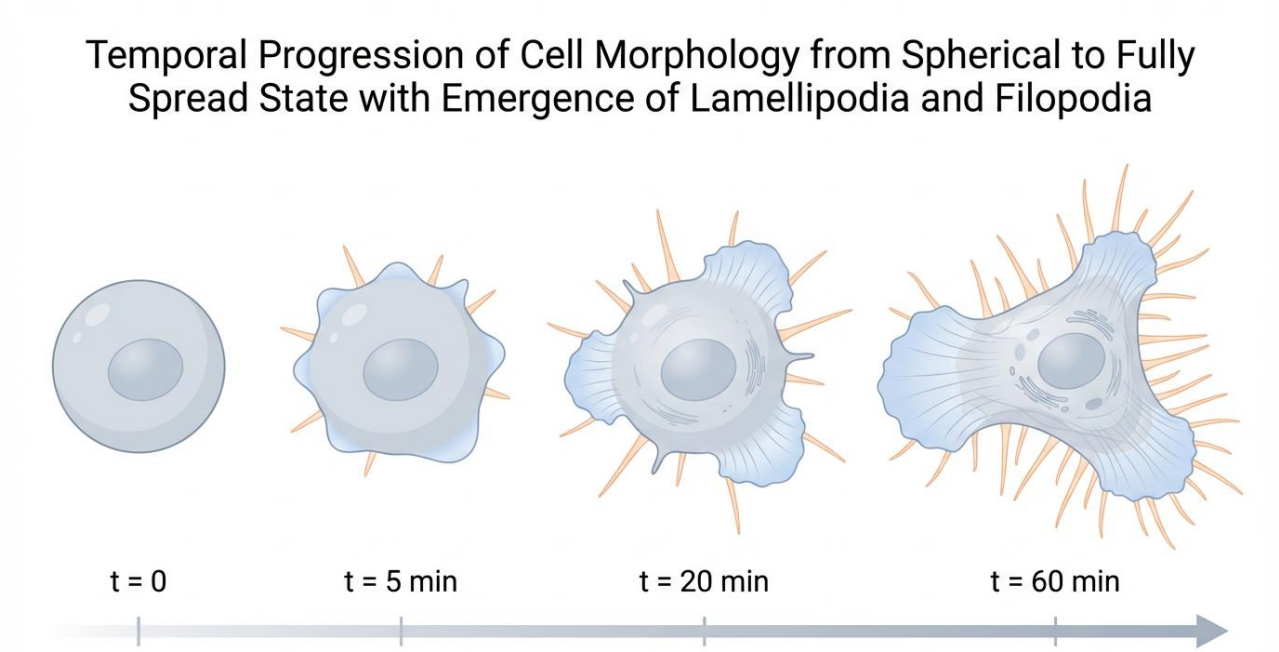
- Adherent cells **are quantified**.

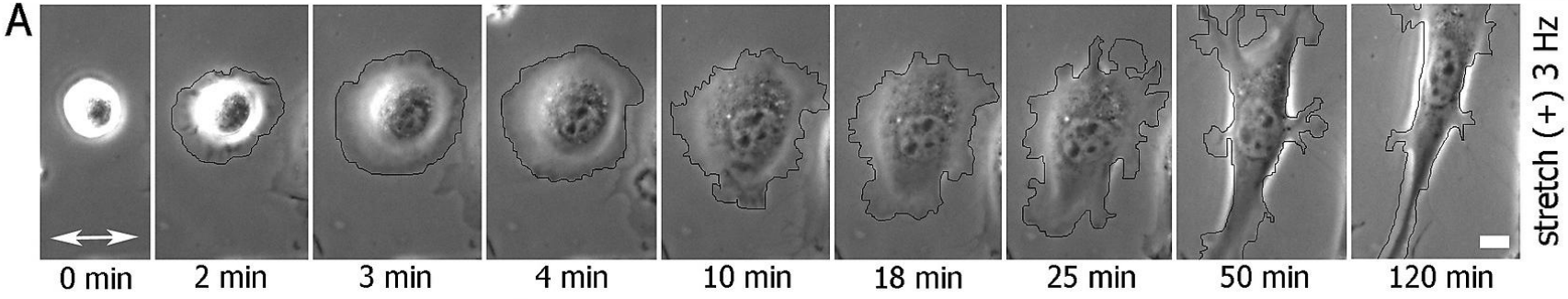


Cell spreading assay

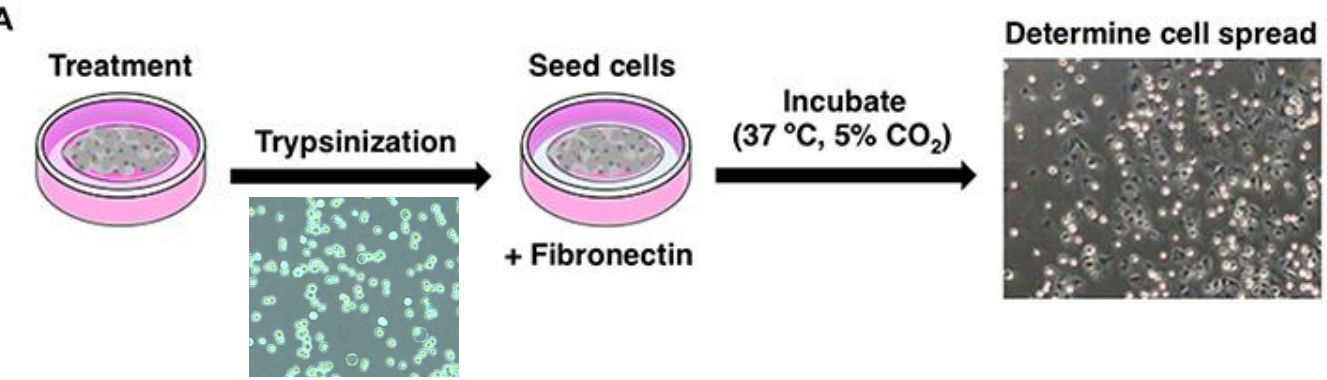
Cell spreading is when cells in its **rounded morphology** in suspension **flatten out** on a substrate.





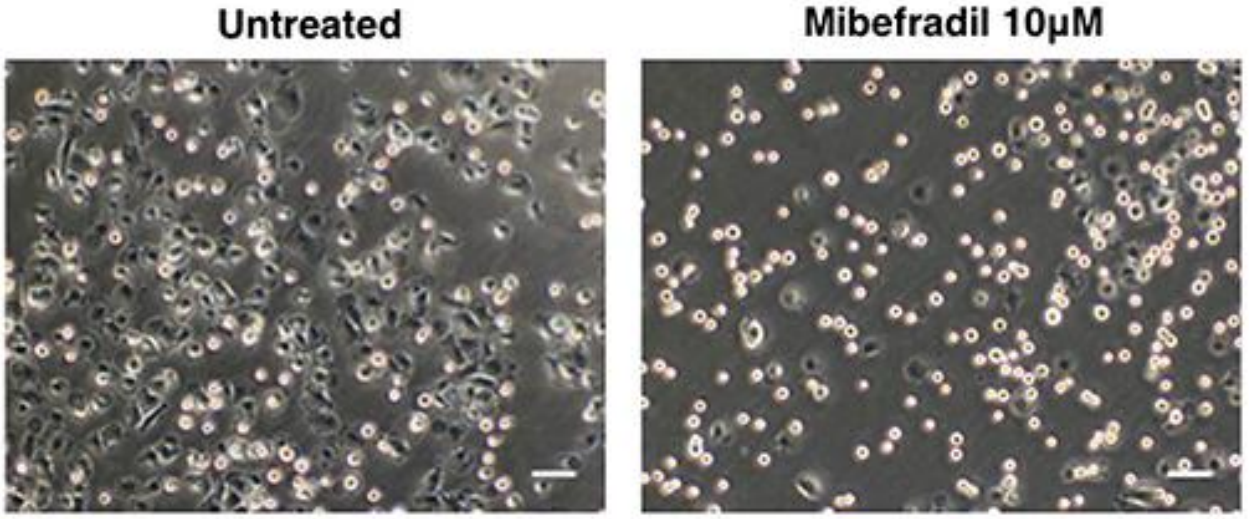


Cell spreading assay



- Cells were treated for 24 h,
- trypsinized
- seeded onto fibronectin coated plate.
-
- **After 1 h cells** were fixed with 2% PFA

- phase-contrast images were captured.



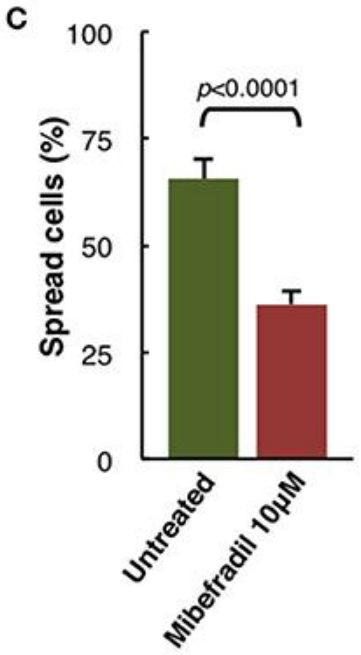
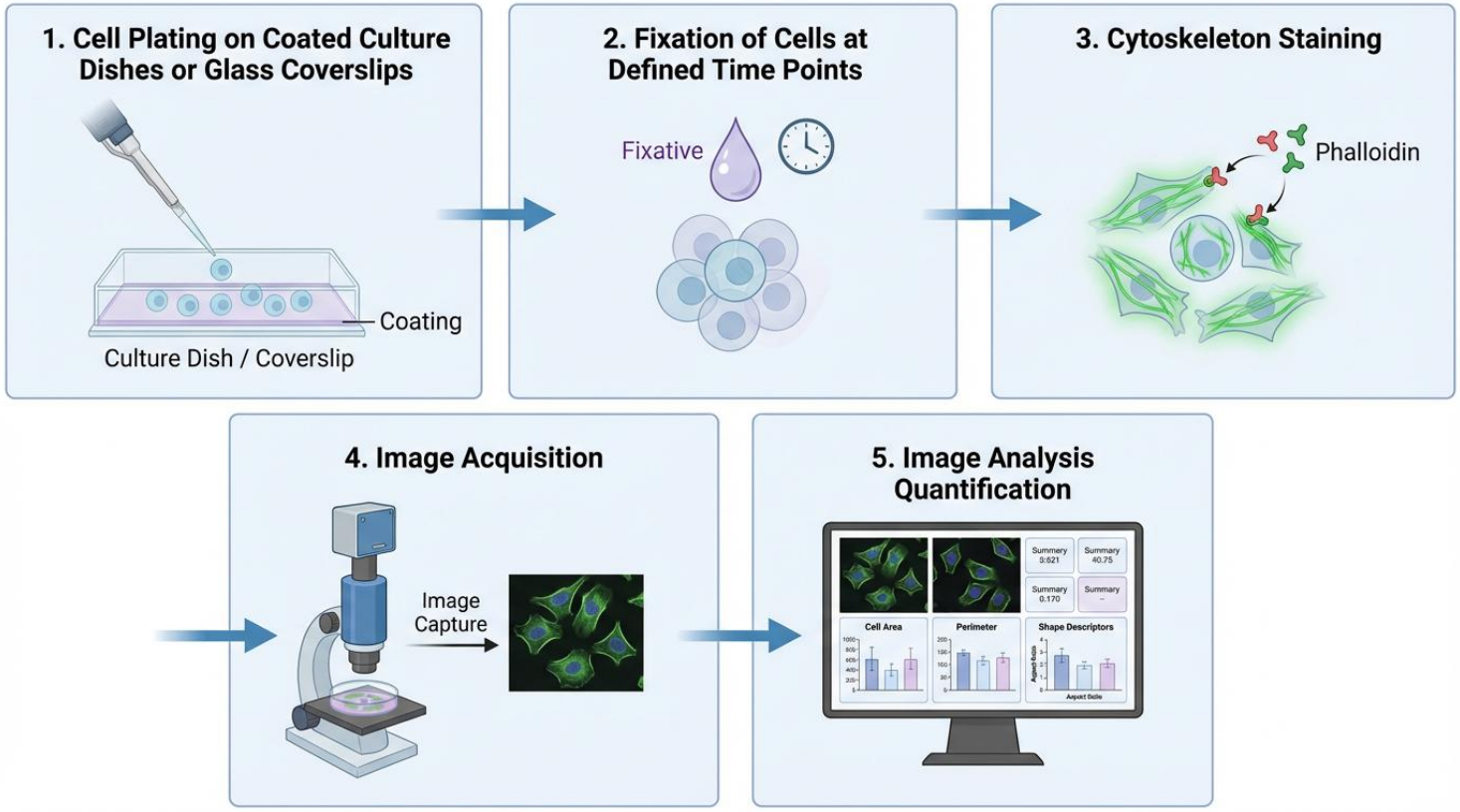
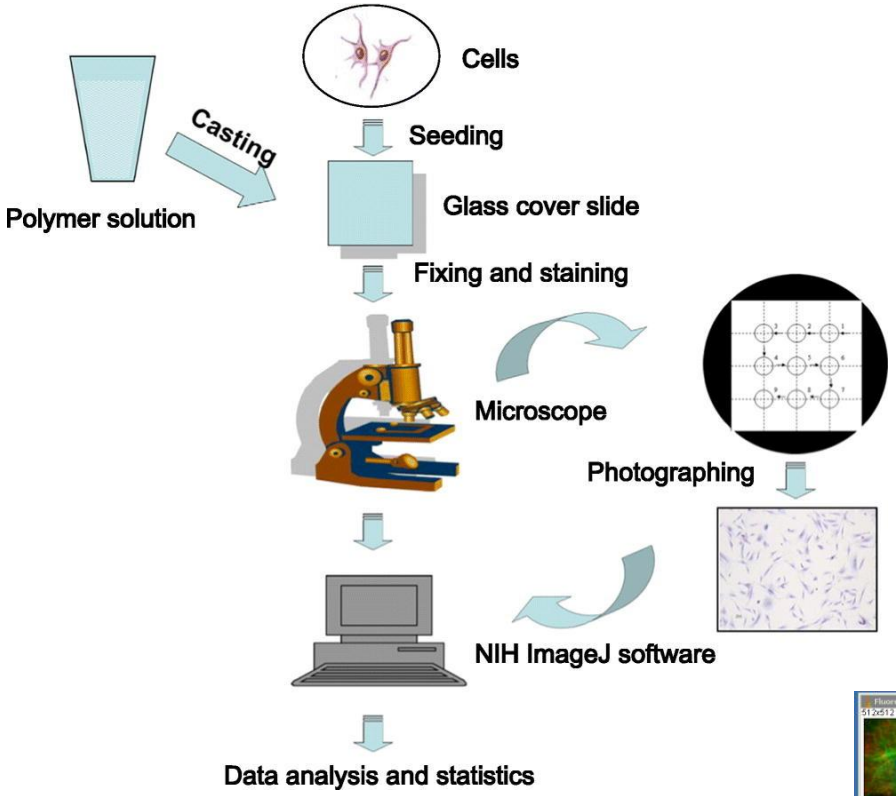
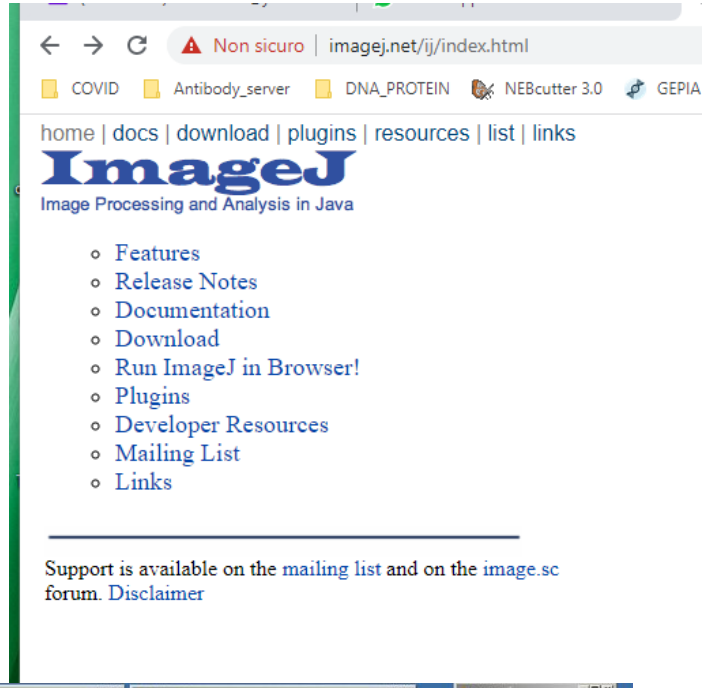
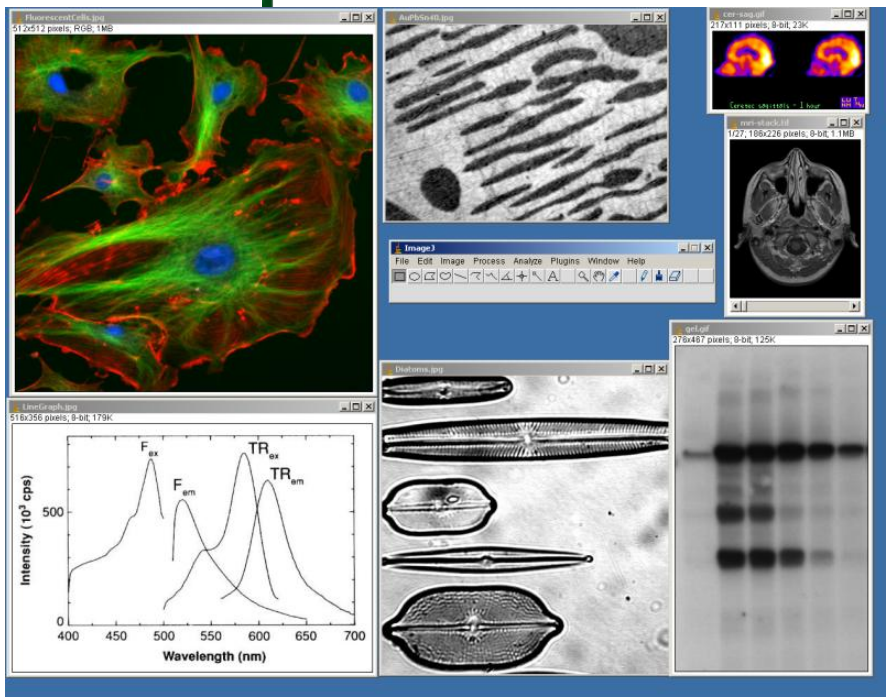


FIGURE 5. (B) phase-contrast images. Scale bars, 50 µm.
C) Plot showing the percentage of spreading cells**. Round bright cells were considered unspread**. Values are percentage of spread cells ± SD (*n* = 3 independent experiments; at least 600 cells for each experiment were counted). The corresponding *p*-value obtained by unpaired two-tailed Student's *t*-test is shown.


1. Cell Plating on Coated Culture Dishes or Glass Coverslips
Coating
Culture Dish / Coverslip
2. Fixation of Cells at Defined Time Points
Fixative
3. Cytoskeleton Staining
Phalloidin
4. Image Acquisition
Image Capture
5. Image Analysis Quantification
Cell Area
Perimeter
Shape Descriptors


Cells
Casting
Seeding
Polymer solution
Glass cover slide
Fixing and staining
Microscope
Photographing
NIH ImageJ software
Data analysis and statistics

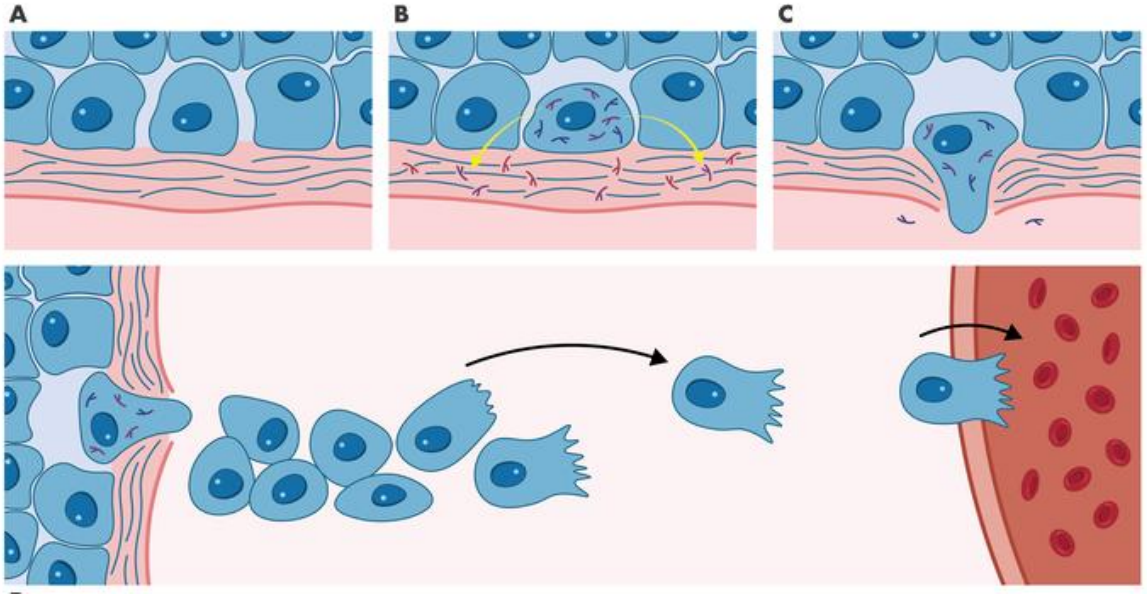

Non sicuro | imagej.net/ij/index.html
COVID
Antibody_server
DNA_PROTEIN
NEBcutter 3.0
GEPIA
home | docs | download | plugins | resources | list | links
ImageJ
Image Processing and Analysis in Java
Features
Release Notes
Documentation
Download
Run ImageJ in Browser!
Plugins
Developer Resources
Mailing List
Links
Support is available on the mailing list and on the image.sc forum. Disclaimer


Intensity (10³ cps)
Wavelength (nm)
F_ex
F_em
TR_ex
TR_em

Early Metastasis Process

The early steps of the metastasis process involve
A) Loosening of the intercellular junctions between tumor cells,
B) Degradation of the intracellular matrix of the basement membrane,
C) Invasion into surrounding tissue,

modeled with the **Invasion Assay.**



D) Migration to endothelial vessels.

modeled with the **Migration Assay.**

Definition of **migration** and **invasion**

Migration is often used as umbrella term in biology to describe **any directed cell movement within the body.**

The ability to migrate **allows cells to change their position** within tissues or between different organs.

In pathology, **invasion** **(of carcinomas)** is defined as

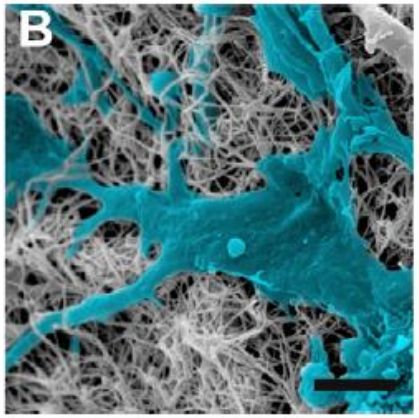
the **penetration of tissue barriers**, and infiltration (intrusion) into the underlying interstitial tissues (by malignant tumor cells)

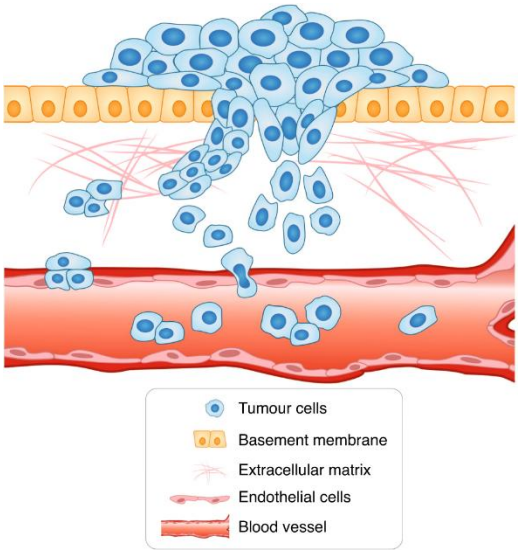
Migration and **invasion** are clearly separated terms in **experimental cell biology**.

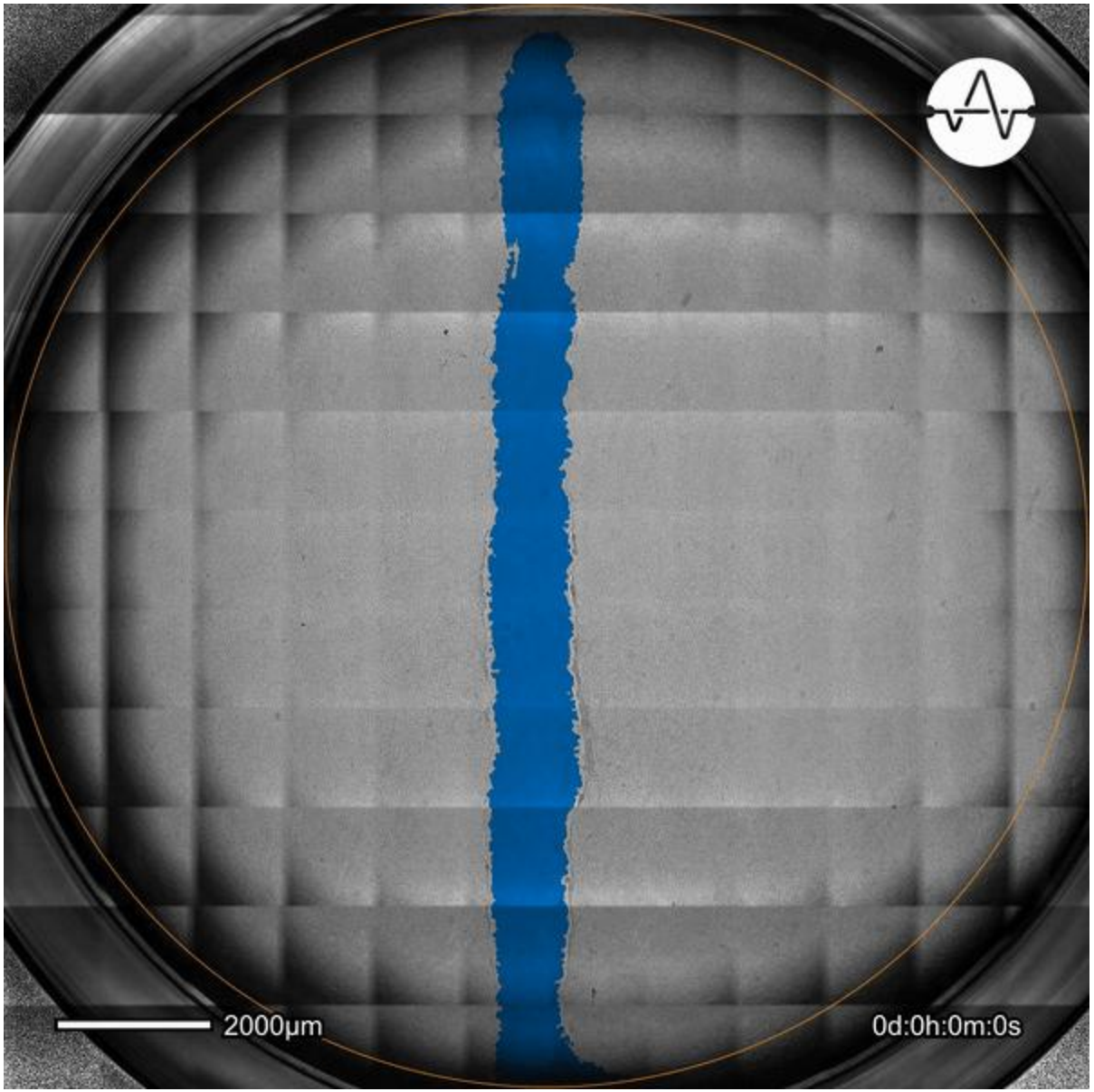
Migration is defined as:
the **directed movement of cells on a substrate** such as basal membranes, ECM fibers or plastic plates.
Therefore, migration **is occurring on 2D surfaces** without any obstructive fiber network

Invasion is defined as:
cell movement through a 3D matrix, which is accompanied by a **restructuring of the 3D environment**.

Invasion requires adhesion, proteolysis of extracellular matrix components and migration, therefore invading cells remodel the ECM.

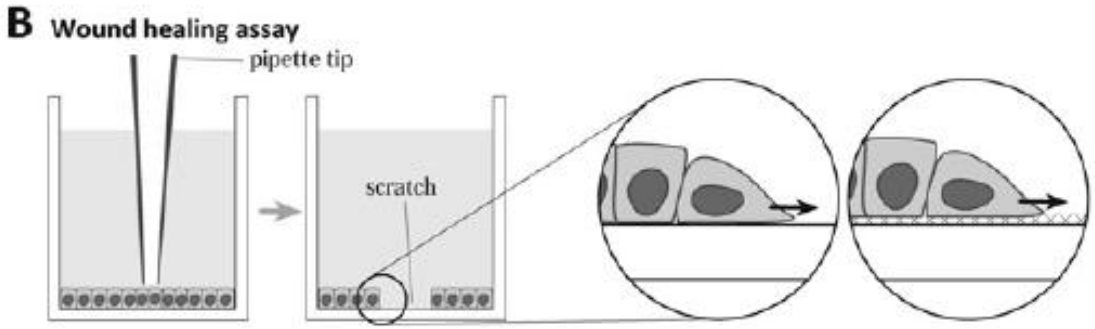




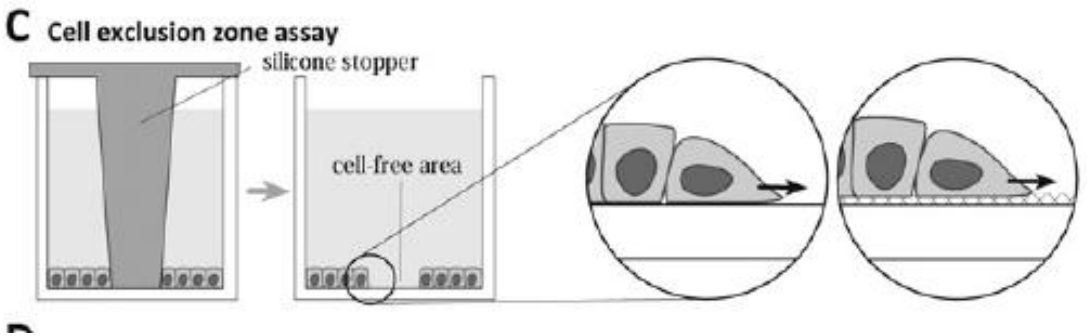

2000µm
0d:0h:0m:0s

Migration Assays

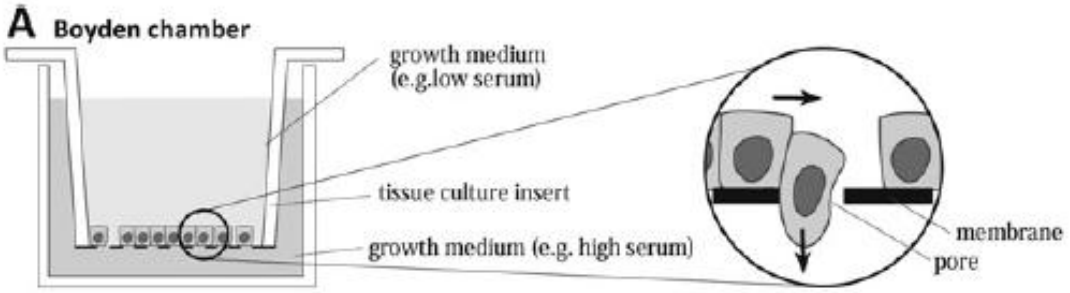
Wound healing assay



Cell Exclusion Zone assay



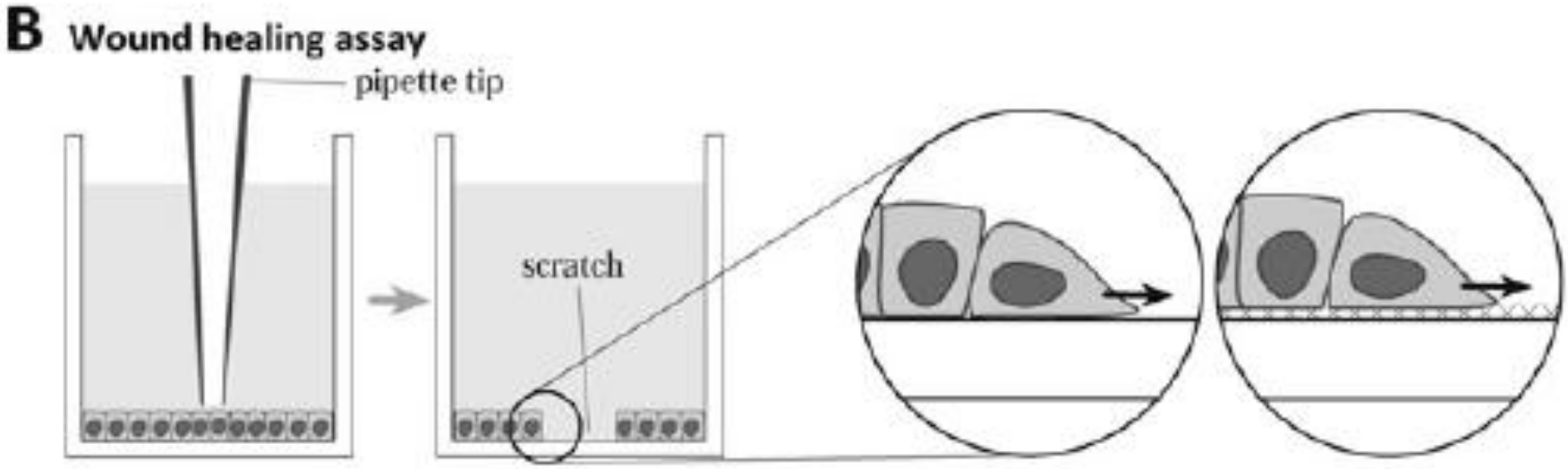
Boyden Chamber



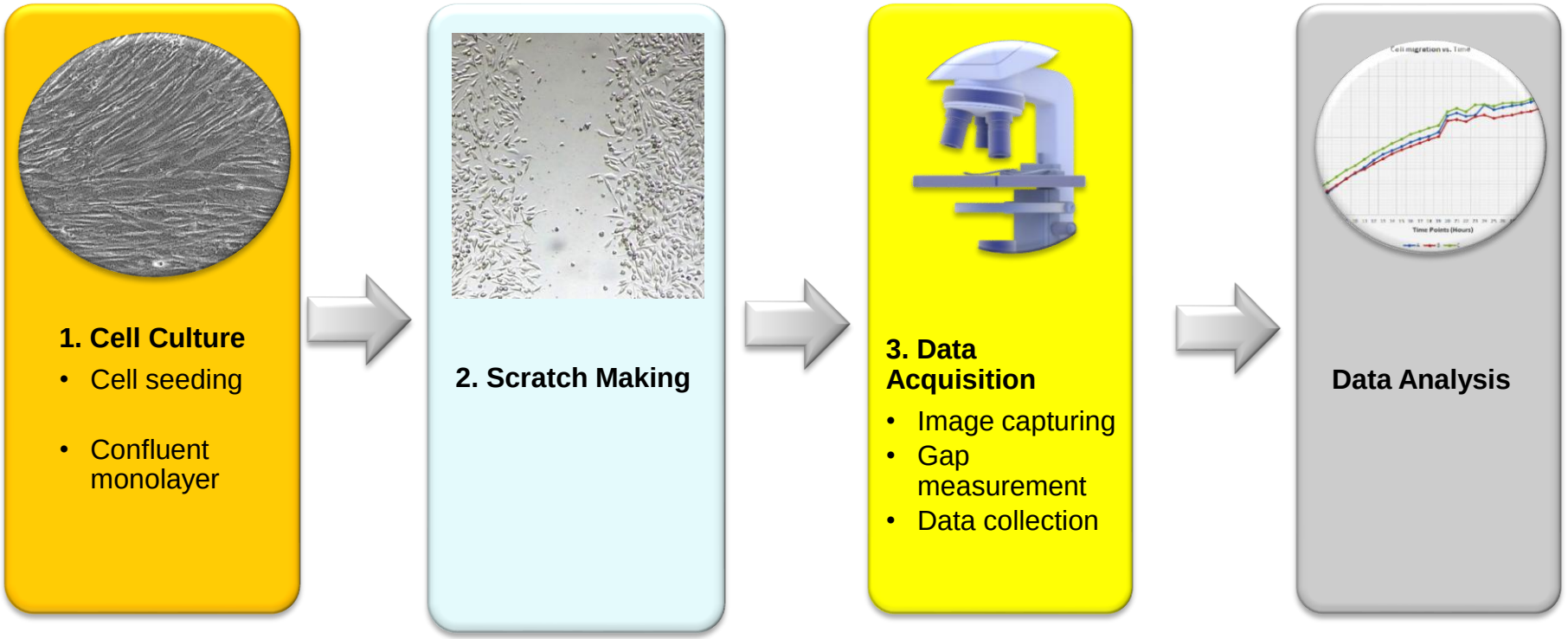
In vitro wound-healing assay (scratch assay)

This popular, technically non-demanding and cheap assay can be used to study **migration of cells on 2D surfaces**.

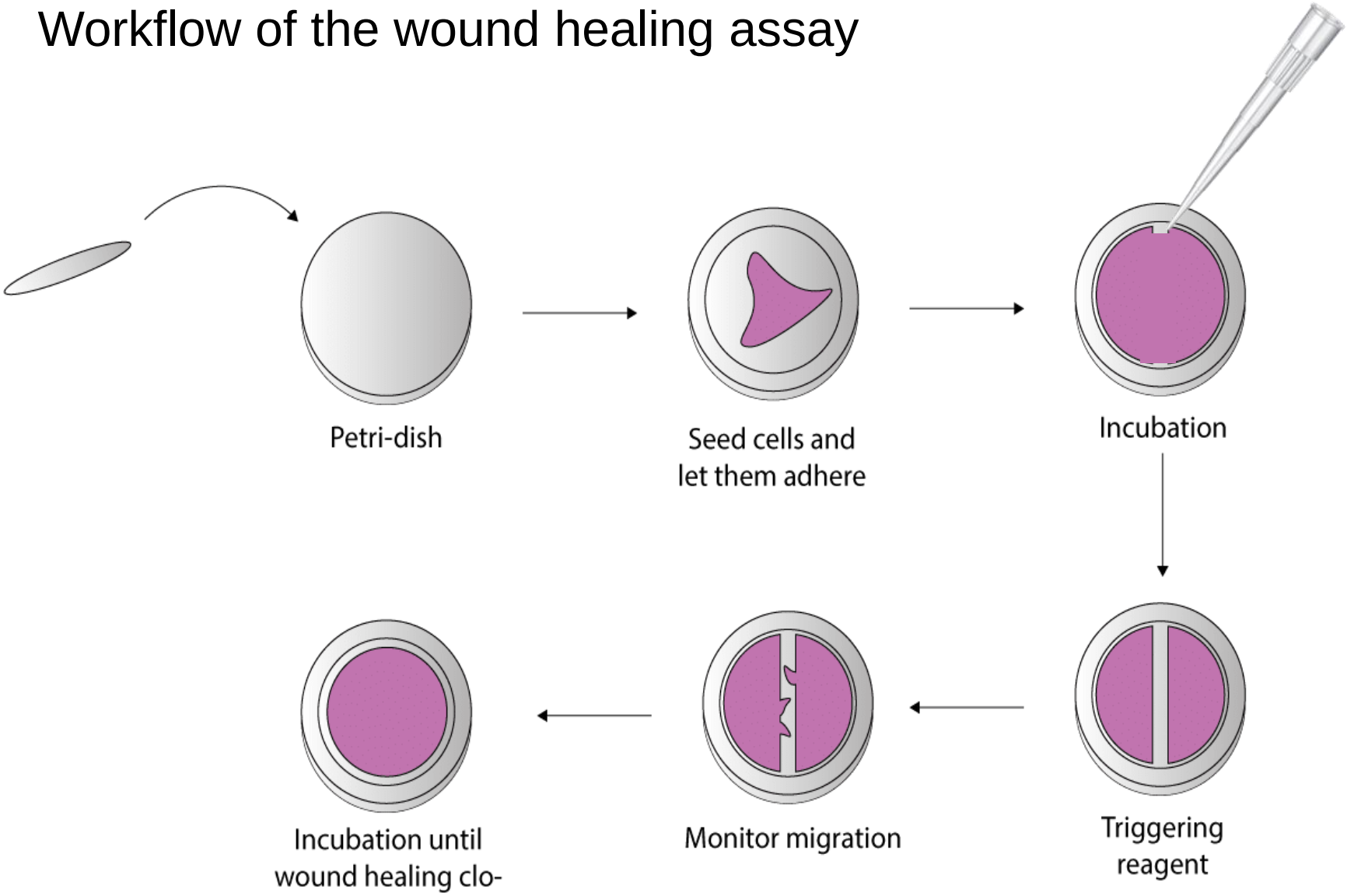
A confluent plate of any type of attached cells is **‘‘wounded’’** by scraping off an area of cells, which is most easily done using **a plastic pipette tip**



Workflow of the **wound healing assay**



Workflow of the wound healing assay



Cell Culture

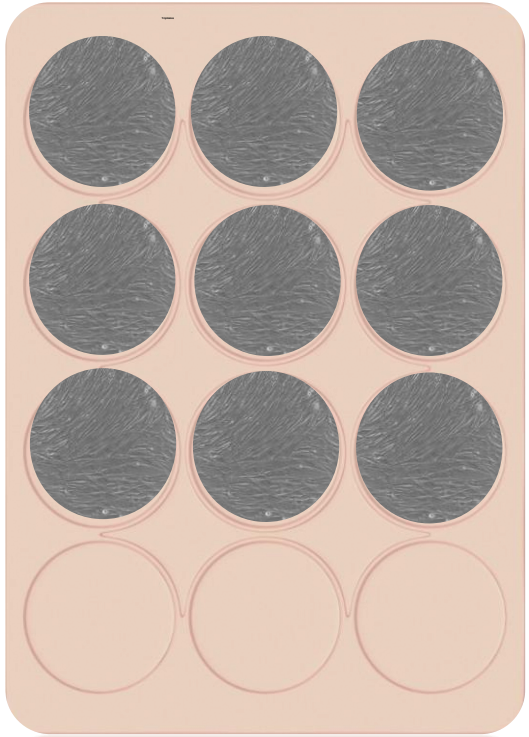
The first step of the assay is to **culture a confluent cell monolayer.**

This monolayer represents the in vivo conditions of the tissue **before wounding** such as, an intact epithelium.

Sample A

Sample B

Sample C



Seed your cells in multi-well plates and incubate until they form near-confluent monolayers

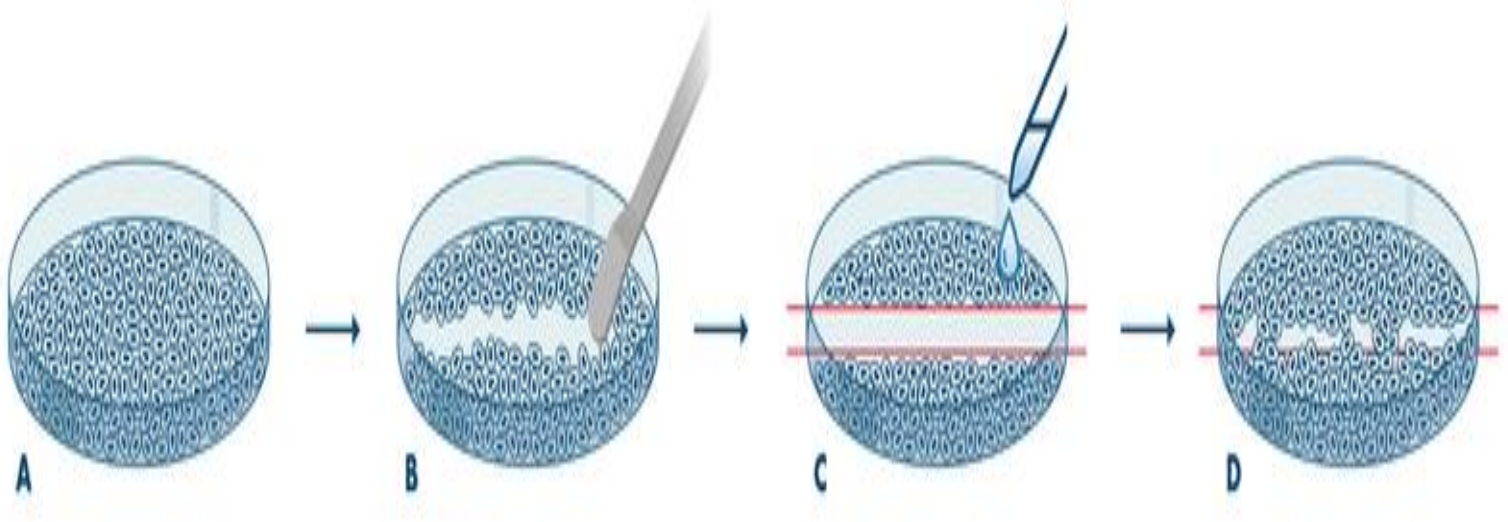
cell proliferation can be a confounding factor.

Cell proliferation can compete with cell migration to fill the gap made during the assay. If this occurs, **the cell medium can be optimized to reduce cell proliferation**.

Decreasing the concentration of serum (**serum starvation**) is the most common change.

Scratch-making

After the cells have become confluent, the next step is to make a cell-free gap in the monolayer. The most frequently used method is to wound the monolayer by mechanical scratching (**scratch wound**) or stamping.

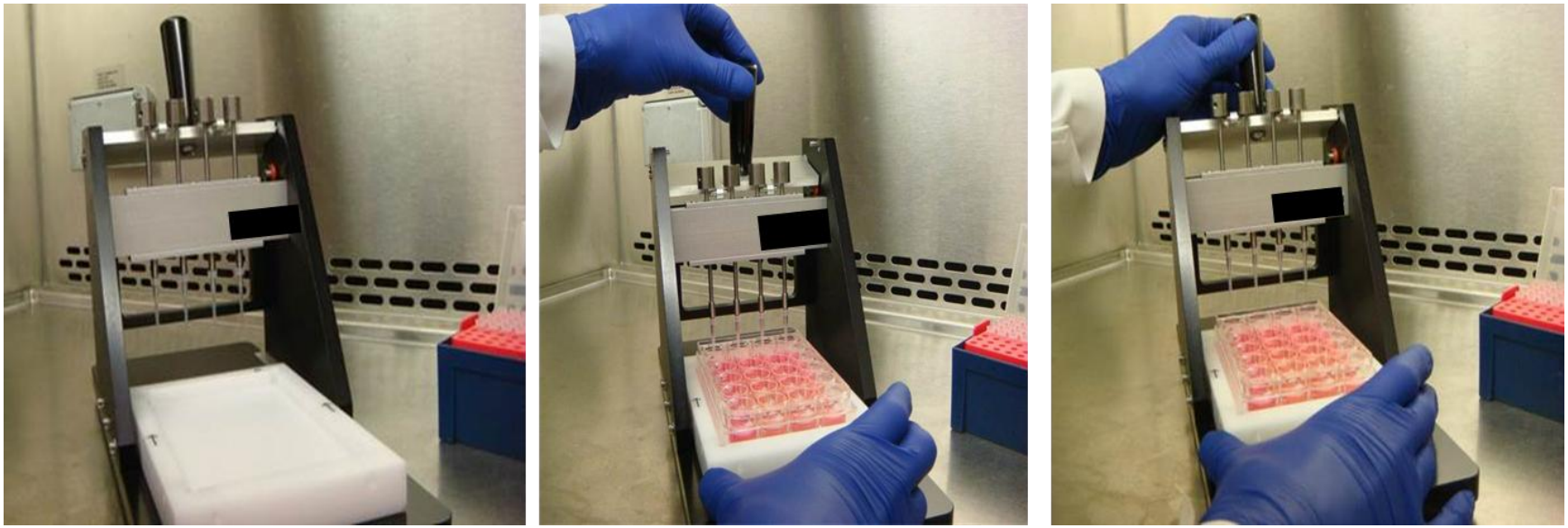


Drawbacks with the manual method of wounding include **low throughput and well-to-well variation of the gap width**.

Scratch-making

This reproducibility can be improved with the use of **commercial tools for making uniform gaps.** Modifications to the wound healing assay that make use of automation can increase both throughput and improve reproducibility.

Ensuring reproducibility is important for the following data acquisition step.

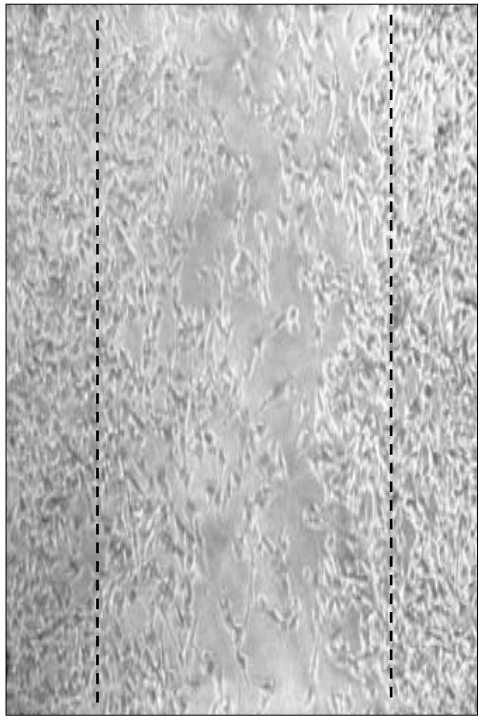


To enhance reproducibility, a scratch-making device creates identical linear scratches in all wells.

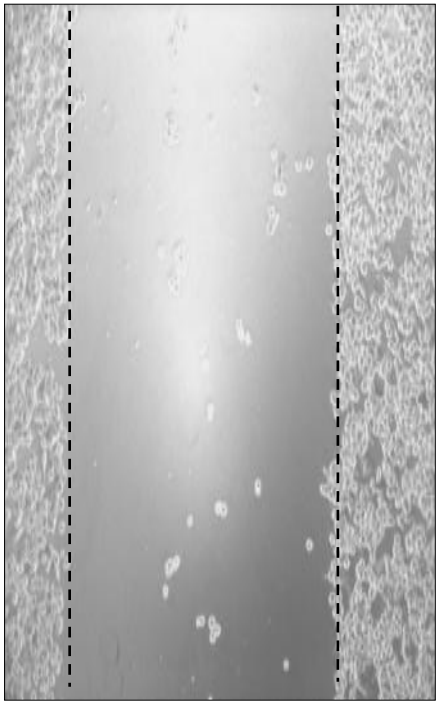
Migration

(Wound Healing Migration Assay)

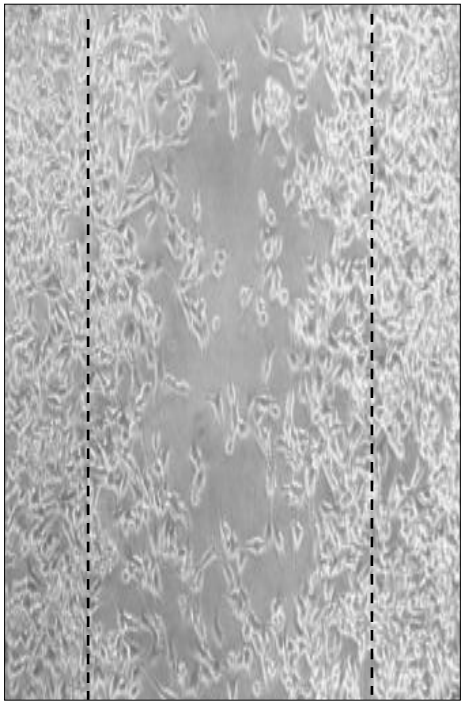
CONTROL



TIME 0

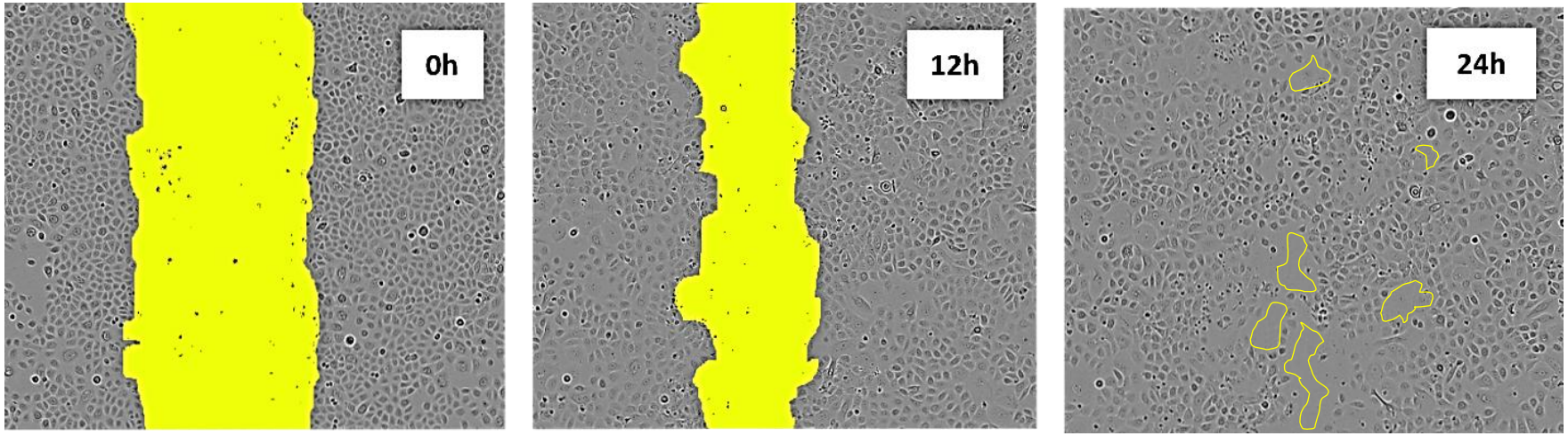


Time 48 Hrs



Data acquisition – scratch images for time-lapse

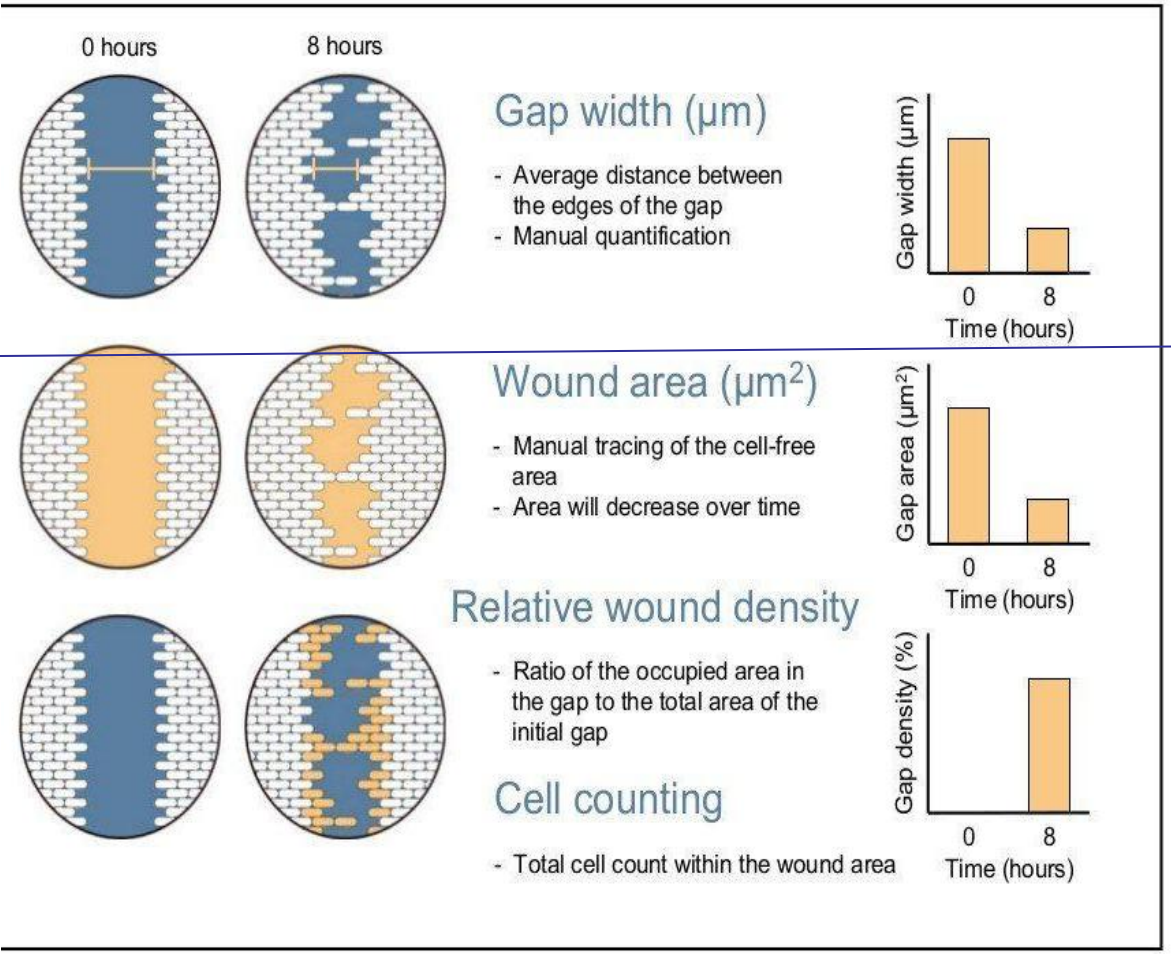
With a cell-free gap prepared, **optical microscopy can be used to observe cells migrating into the wound area**.
Cell migration is best viewed using phase-contrast imaging rather than fluorescence,



Collective migration of human skin cells *in vitro.* Example of images captured during a wound healing experiment. Keratinocyte monolayers were scraped with a pipette tip **and images were taken at 0, 12, and 24 h using time-lapse photography** as the cells repopulate the scratch area “wound” in the center. **The yellow color** indicates the scratch area

Data Analysis

Once images of the gap closing have been acquired, several methods of analysis can be used to quantify the rate of cell migration.



The first method measures **the change in the wound width**

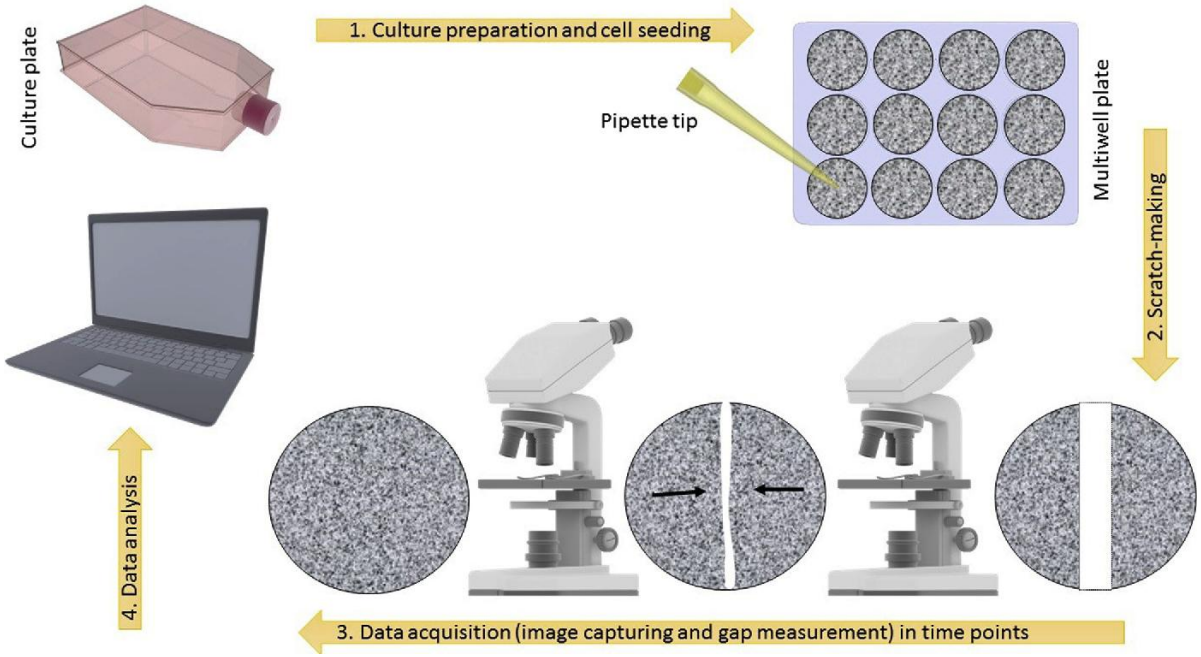
The second method calculates **the change in wound area over time** as a percentage of wound closure.

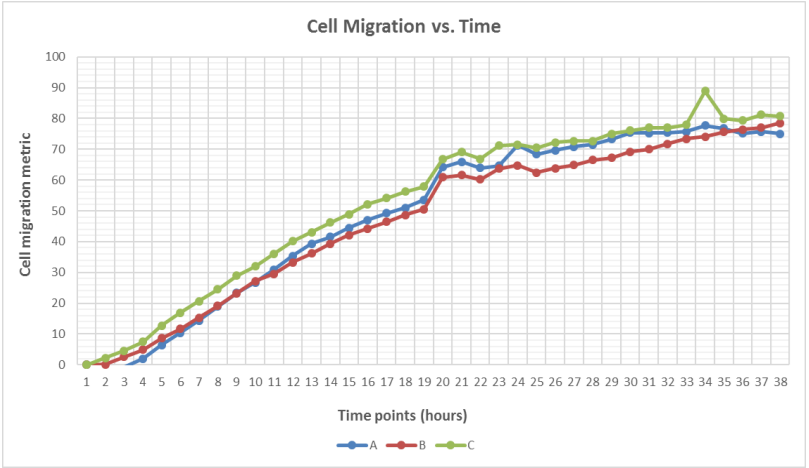
The final method measures the **relative wound density** over time expressed as a percentage

Data acquisition – scratch images for time-lapse

A series of **time-lapse images** (snapshot method) can be acquired as cells migrate into the cell-free gap.

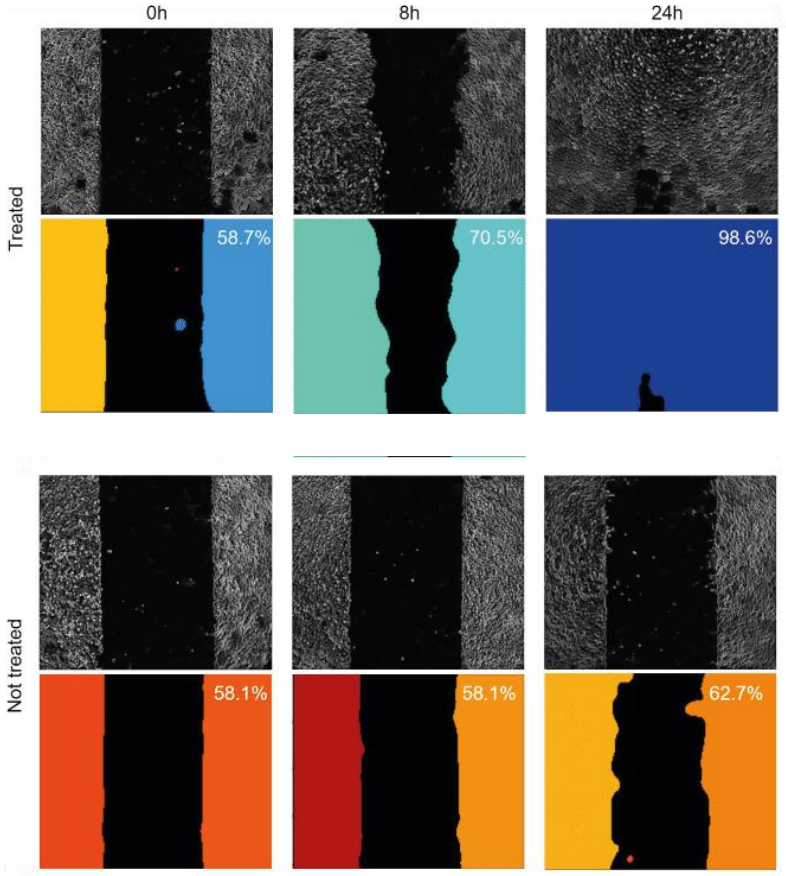
These time points should be collected **within 24 hours of the experiment being started** to minimize the confounding effects of cell replication on gap closure.





Applications

- **Qualitative and Quantitative** analysis of collective **cell migration** under different experimental conditions.
- Studying the effects of **cell-matrix** and **cell-cell** interactions on cell migration.
- High-throughput platforms for genes involved in cancer cell migration, **small molecule screening and drug discovery.**



Advantages

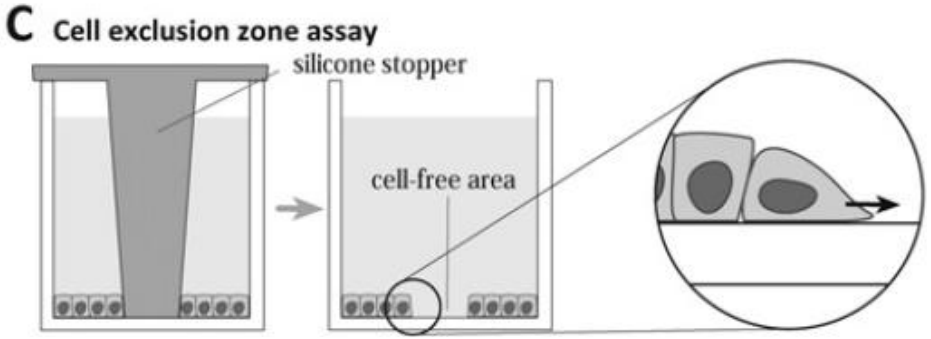
- Relatively **inexpensive and easy** to perform
- Can visually observe cell movement and morphology throughout the experiment
- Testing conditions can be easily adjusted for different purposes
- Creates a strong directional migratory response
- Ability to coat assay surface with an appropriate extracellular matrix (ECM)
- Suitable for **endpoint** and **kinetic assays** and amenable to high throughput screening platforms

Limitations

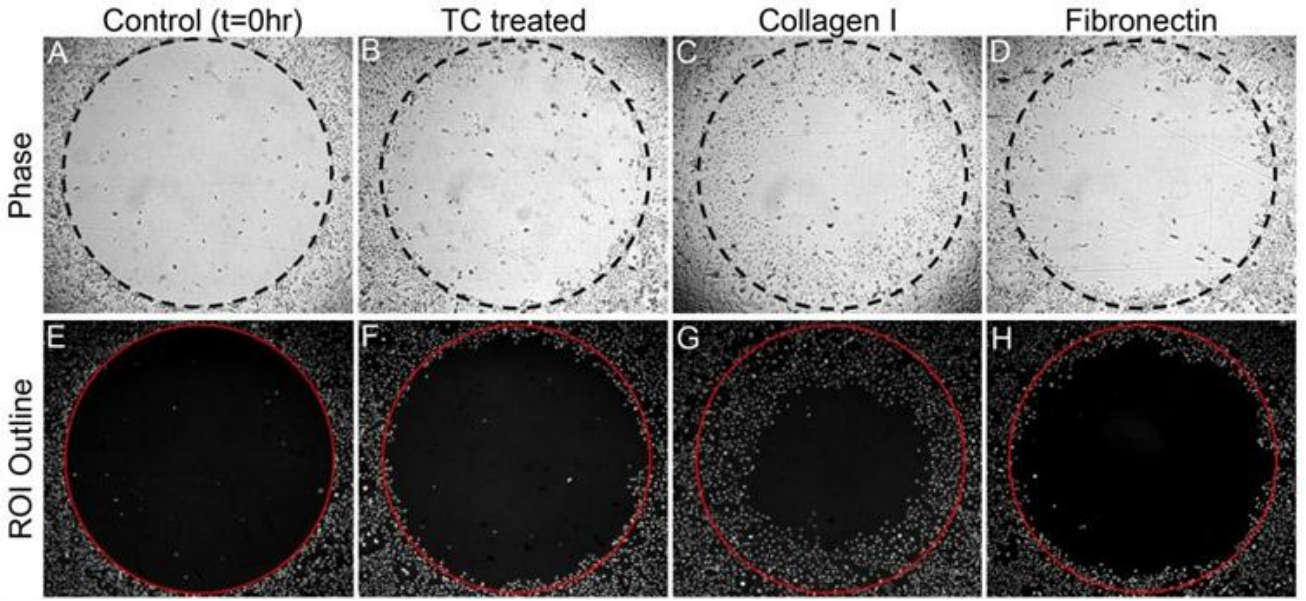
- **May not be suitable for studying specialized primary cells** since a relatively large number of cells is required for the assay (performed in a tissue culture dish)
- Not suitable for chemotaxis studies or for non-adherent cells
- **Lack of standardization** in its application makes it difficult to reproduce experiments, especially when using **manual scratching**
- Scratching introduces **mechanical injury** to the cells, leading to release of cellular contents into the surroundings and potentially influencing the migration process
- **Cell proliferation** may confound the measurement of migration. Therefore, suppression of proliferation is a necessary intervention.

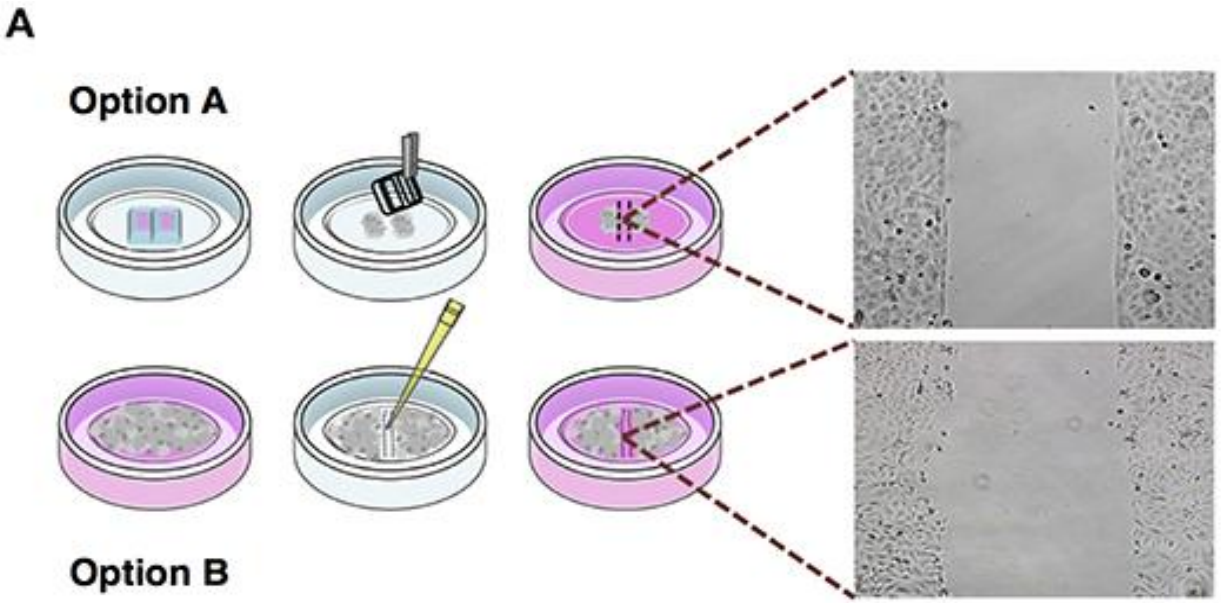
Cell exclusion zone assay

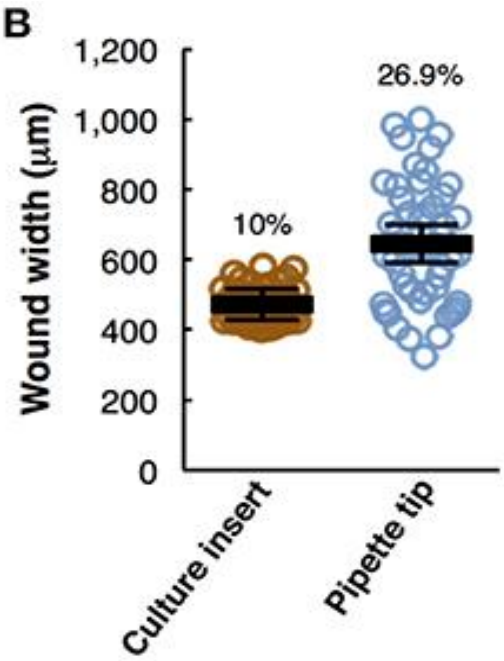
A possibility to circumvent the problem of cell remnants is to create **cell exclusion zones** at the time of cell seeding with e.g. microstencils



These stoppers are positioned prior to seeding of the cells and create an **exclusion zone** with the tip of the stopper







Video Wound healing

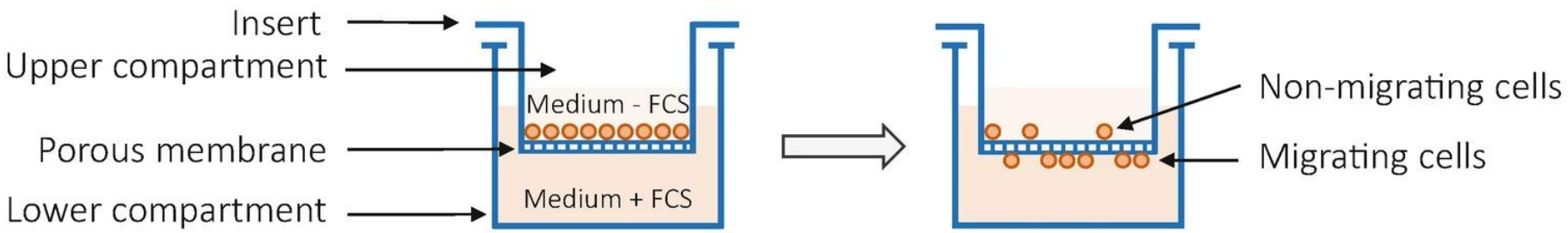
TEC_CELL_02_Cell Migration_IBI_Wound

Transwell migration assay **(Boyden** chamber assay)

The **transwell assay** was originally introduced by **Boyden** (and is therefore often called **Boyden chamber** assay) to analyze the chemotactic responses of leukocytes.

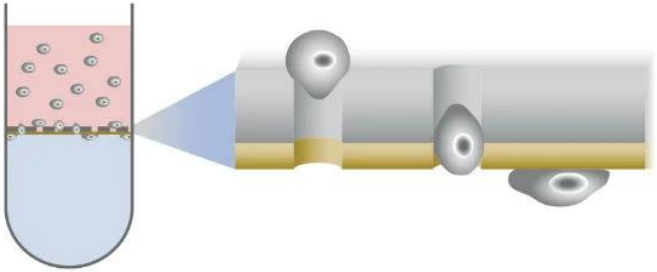
Improved, simplified and disposable versions of the original chambers were developed.

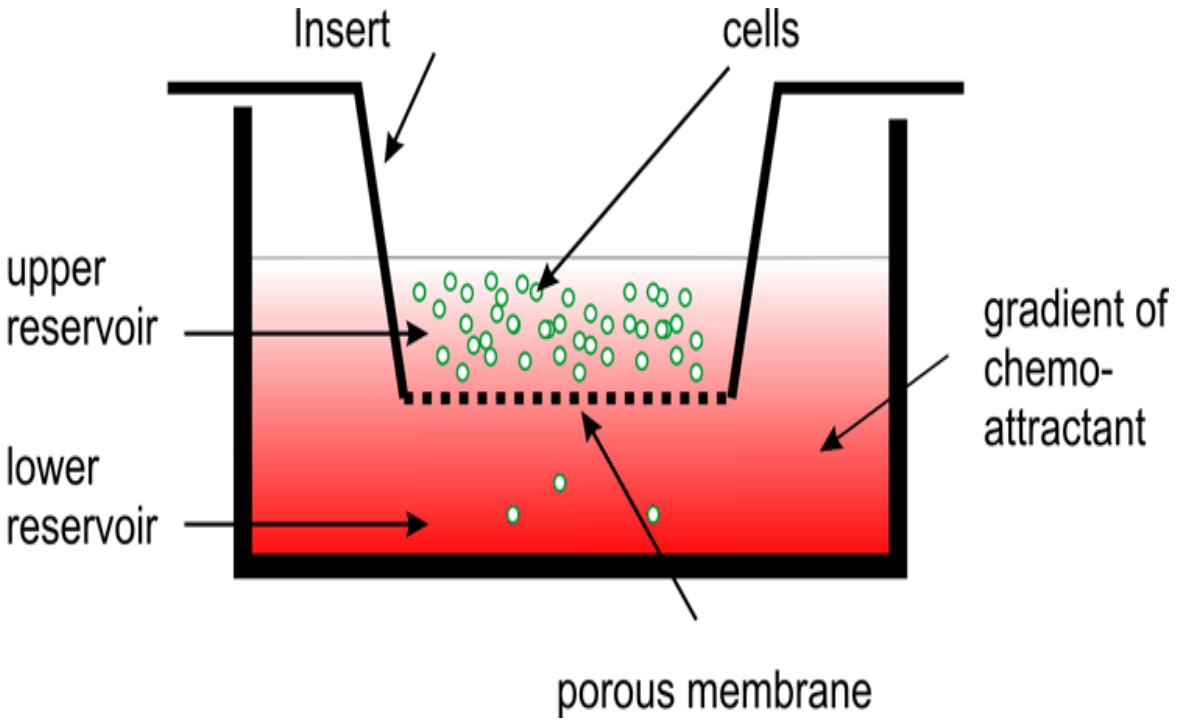
A



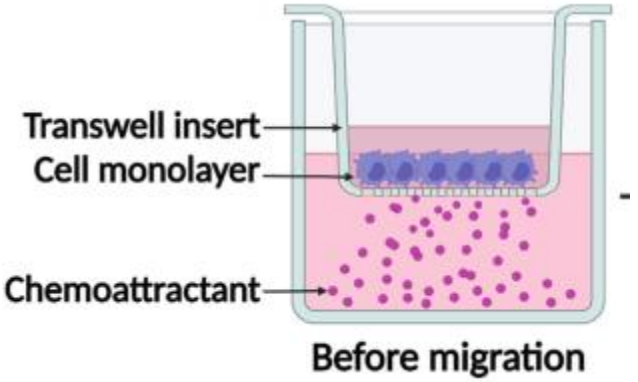
The principle of this assay is based on **two medium containing chambers** separated by a porous membrane through which cells transmigrate.

The size of the cells to be analyzed, determines the required **pore size of the membranes**.

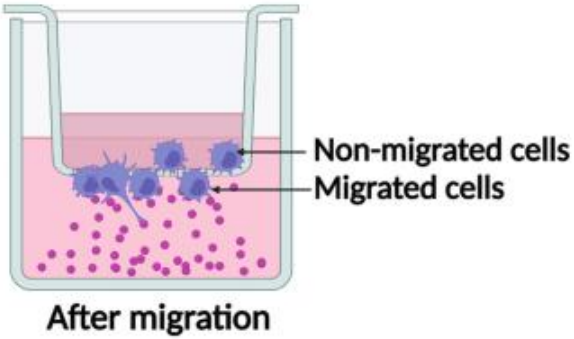




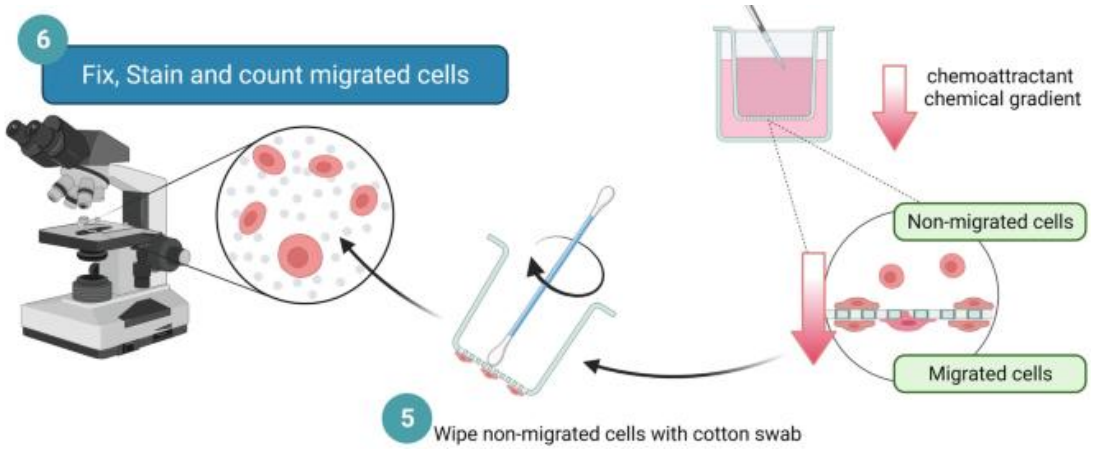
Cells are seeded in medium in the upper part

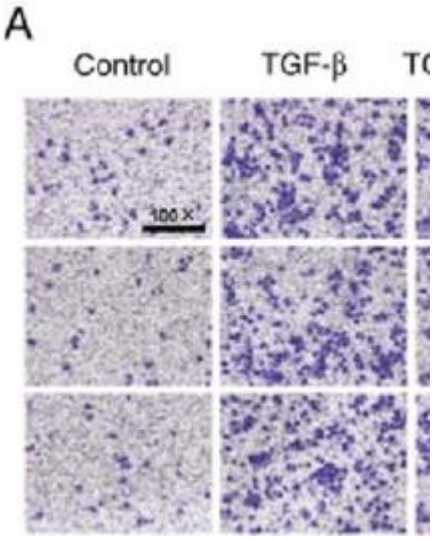


- **migrate in vertical direction** through the pores of the membrane into the lower compartment, in which medium containing an attractant or simply higher serum content is present.



Cell are **stained** and **counted**





The **advantages** of the method are:

the broad availability of different cell culture inserts and sizes;

the relative ease of the experimental setup and the – albeit only short – **medium or cytokine/chemokine gradient** between the upper (cell culture insert) and lower (culture vessel) growth medium.

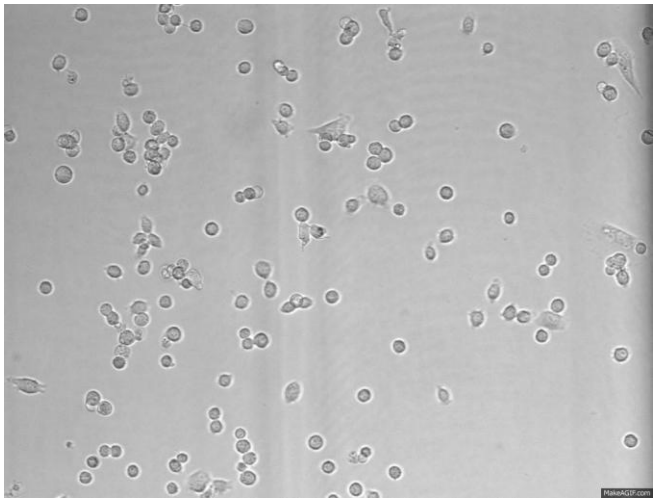
there are some **drawbacks**

This method is an **endpoint assay** if simple cell staining procedures are used.

Non-invaded cells, which stayed on the upper side of the transwell insert, have to be eliminated prior to staining of the invasive cells at the bottom of the membrane.

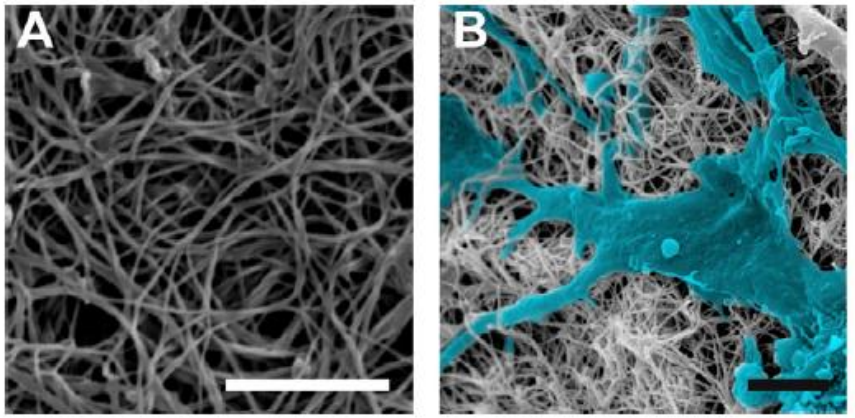
Migration and **invasion** are clearly separated terms in **experimental cell biology**.

Migration is defined as:
the **directed movement of cells on a substrate** such as basal membranes, ECM fibers or plastic plates.
Therefore, migration **is occurring on 2D surfaces** without any obstructive fiber network



Invasion is defined as:
cell movement through a 3D matrix, which is accompanied by a
restructuring of the 3D environment.

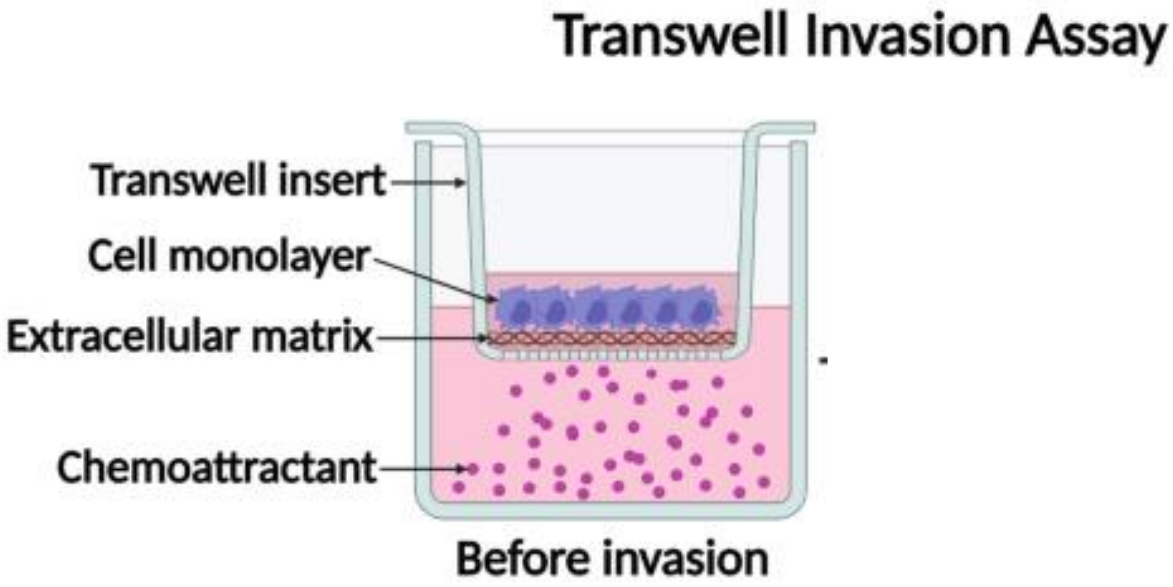
Invasion requires adhesion, proteolysis of extracellular matrix components and migration, therefore invading cells remodel the ECM.



Transwell invasion assay

The principal technical setup of the transwell invasion assay equals the transwell migration assay.

In addition, the porous filter **is overlaid by a thin layer of ECM**, before seeding the cells into the top chamber .



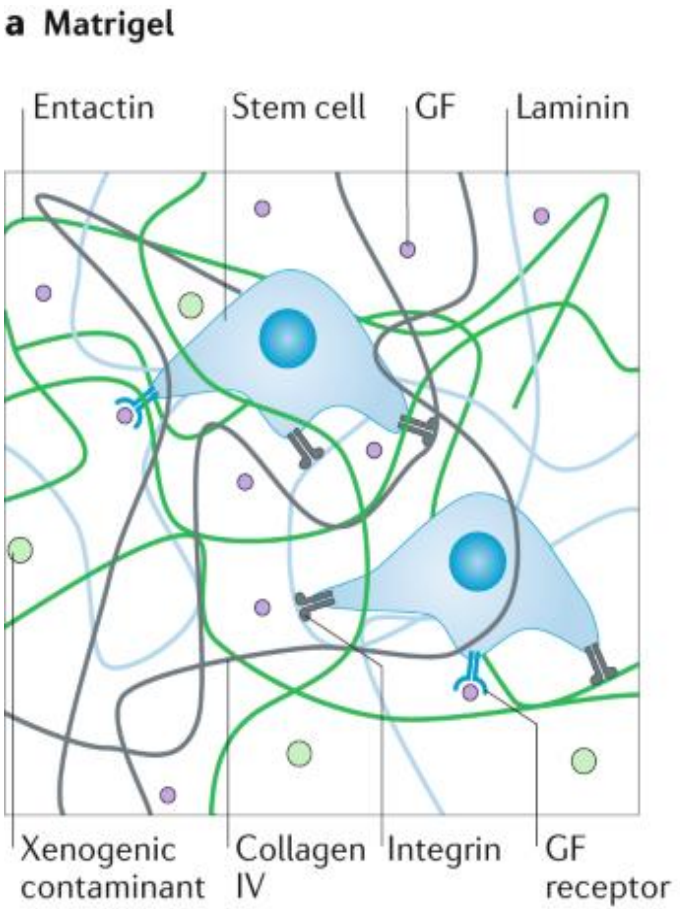
Corning **Matrigel** matrix is a solubilized basement membrane preparation extracted from the Engelbreth-Holm-Swarm (EHS) mouse sarcoma, a tumor rich in extracellular matrix proteins

The primary components of Matrigel are four major basement-membrane ECM proteins:

- **laminin** (~60%),
- **collagen IV** (~30%),
- entactin (~8%)
- heparin sulfate proteoglycan perlecan (~2–3%)

Matrigel also contains tumour-derived proteins, including growth factors

During preparation, the reconstituted form of Matrigel **undergoes gelation at temperatures in the range 22–37 °C**, during which entactin acts as a crosslinker between the laminin and collagen IV to **create a hydrogel** — a water-swollen, crosslinked network.

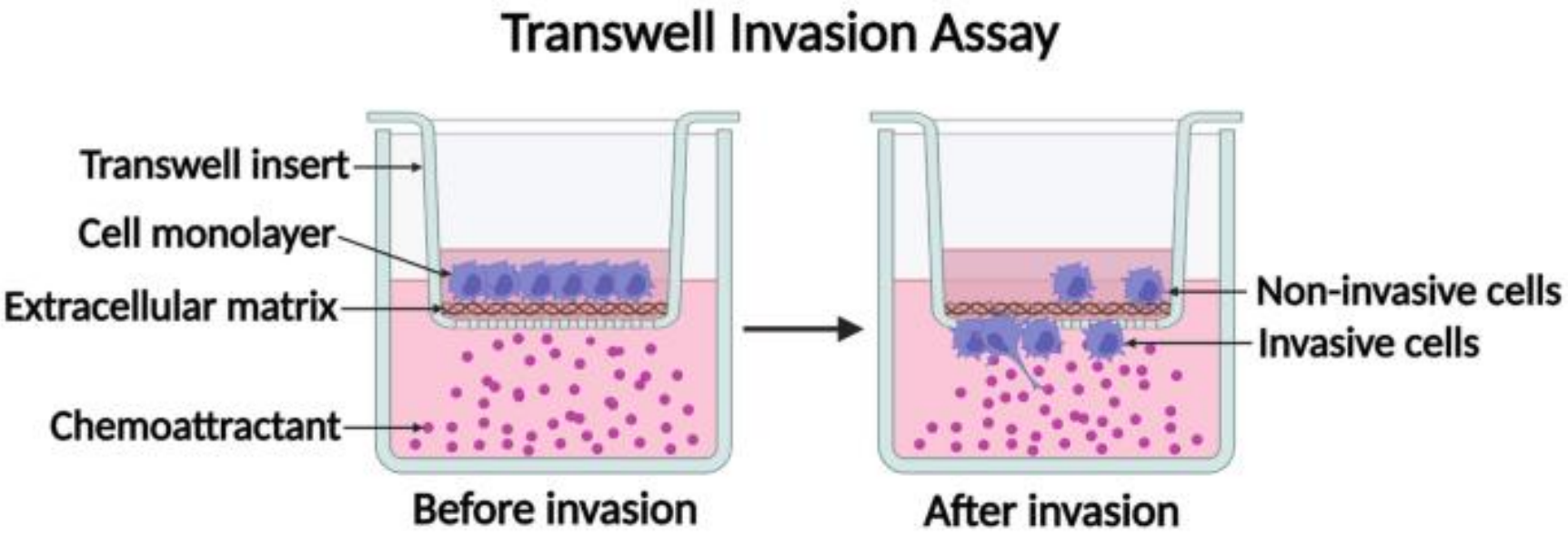


Application

Cell Growth and Differentiation
Metabolism/Toxicology Studies
Invasion Assays
in vitro and in vivo Angiogenesis Assays
in vivo Angiogenesis Studies and Augmentation of Tumors in Immunosuppressed Mice

The **ECM occludes the membrane pores,** blocking non-invasive cells from migrating through.

By contrast, **invasive cells can degrade the matrix and move through the ECM** layer and adhere to the bottom of the filter



- Matrigel basement membrane matrix

- Collagen I
- Collagen IV

- Fibrinogen
- Fibronectin

- Laminin

- Bovine Serum Albumin (BSA)

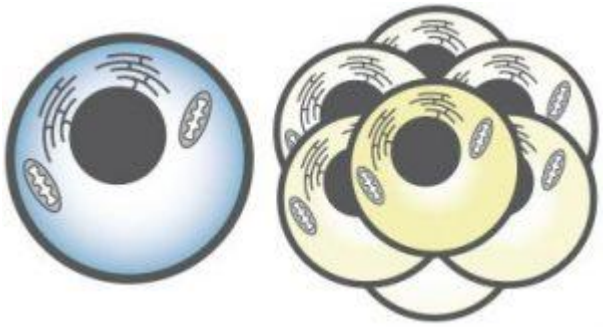
Viability and Proliferation Assays

Viability and **proliferation** are two distinct characteristics of cells.

Viability is a measure of the **number of living cells in a population**

proliferation is a measure of cell division.

It should be noted that not all viable cells divide. Although proliferation can readily be interpreted as viability, absence of proliferation should not automatically be taken as a sign of cell death.



MTT Cell Proliferation Assay

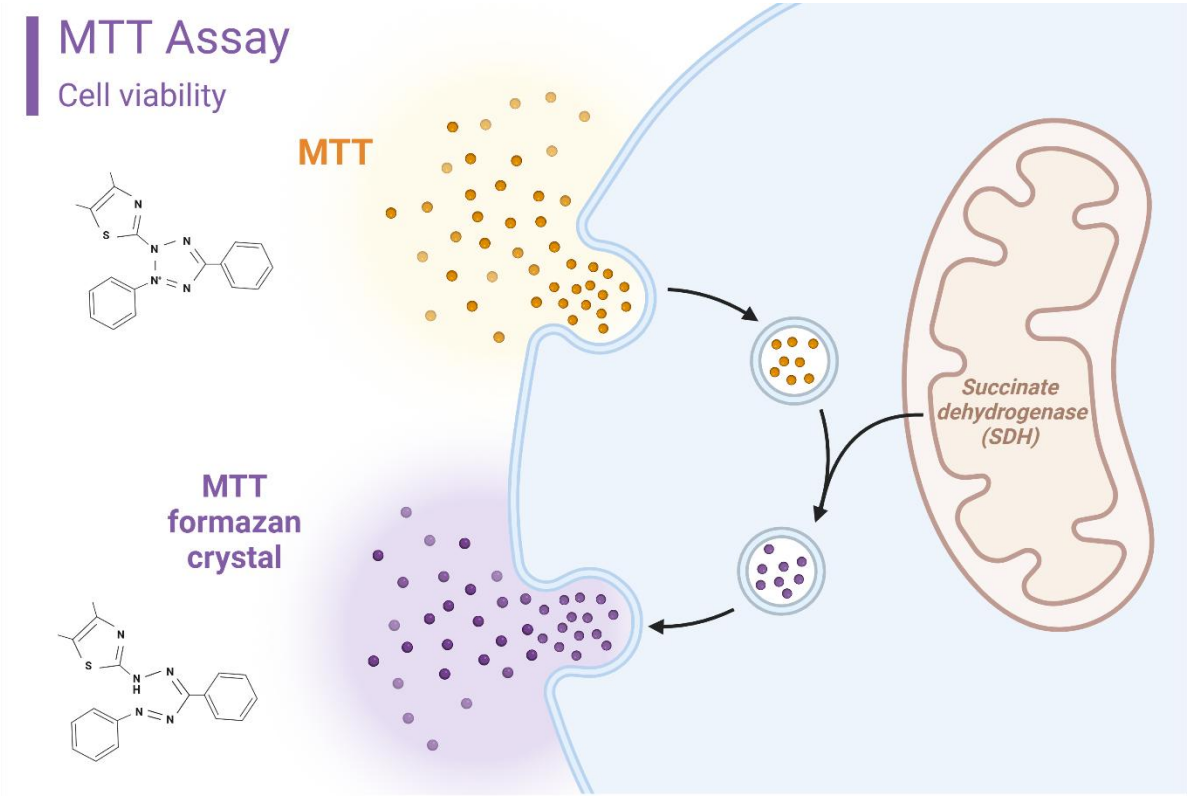
Measurement of **cell viability and proliferation** forms the basis for numerous in vitro assays of a cell population's response to external factors

The yellow tetrazolium MTT

(3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide)

is reduced by metabolically active cells into

intracellular **purple formazan**

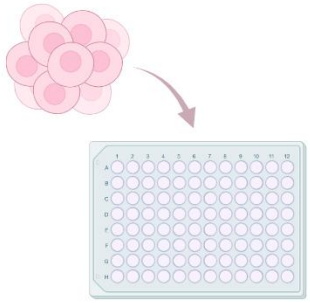


The **reduction of tetrazolium salts** is now widely accepted as a reliable way to examine cell proliferation.

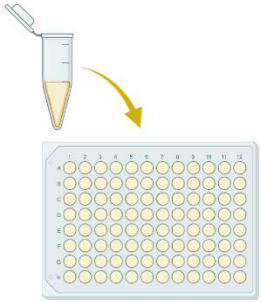
MTT Assay

Cytotoxicity evaluation

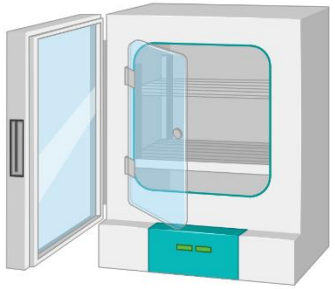
1. Cell seeding in well plate



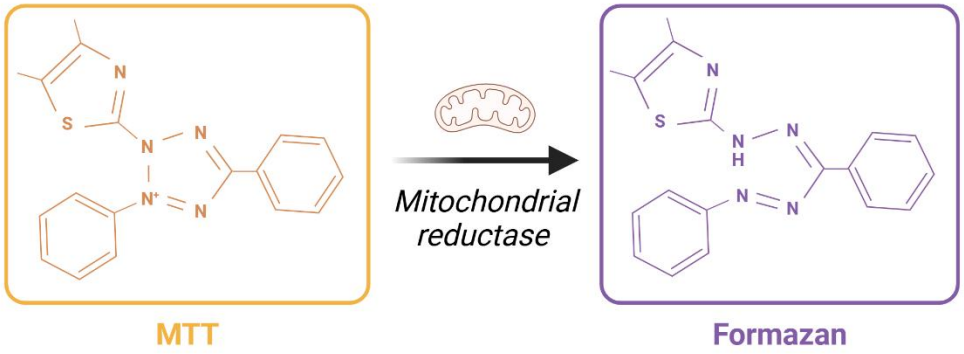
2. MTT reagent addition



3. Incubation for 3-4 hours



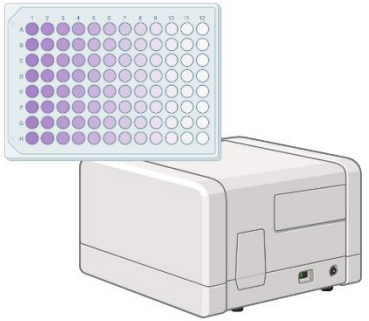
4. Colorimetric reaction to determine cell viability



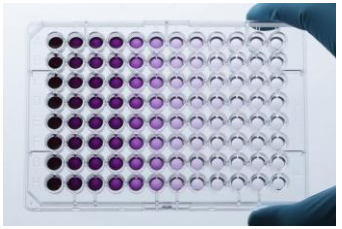
MTT

Formazan

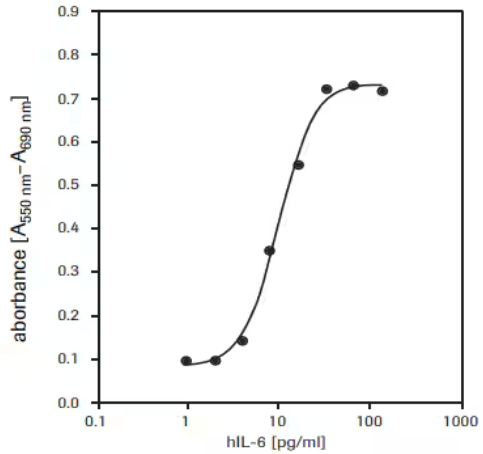
5. Absorbance reading



The **MTT Cell Proliferation Assay** measures:

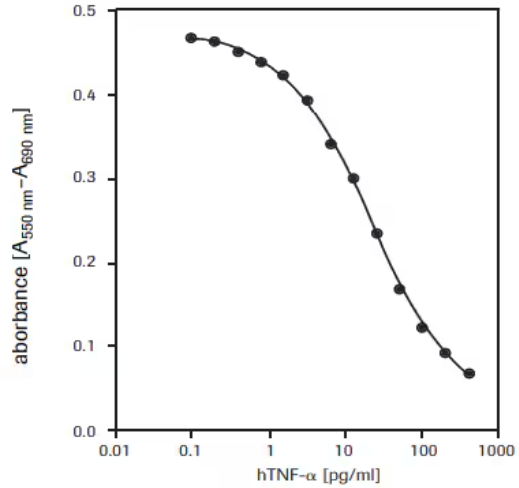


- the **cell proliferation rate**



PER MISURARE LA CRESCITA CELLULARE

- conversely, when metabolic events lead to apoptosis or necrosis, **the reduction in cell viability**



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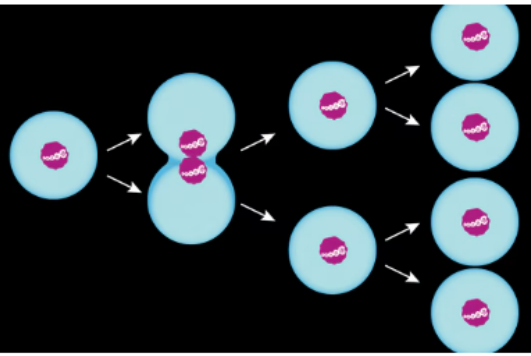
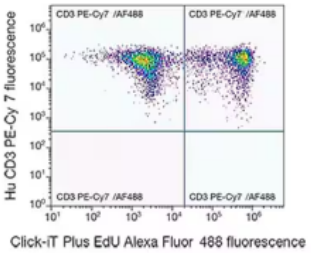
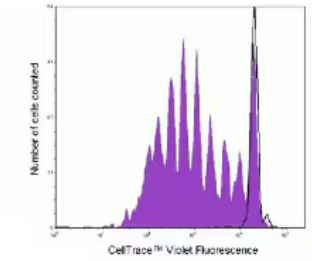
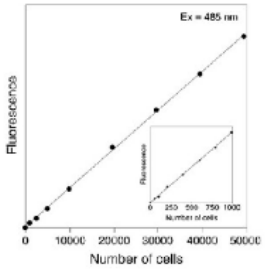
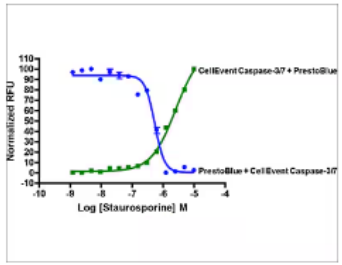
Cell Proliferation Assays

Assays and reagents to evaluate cellular proliferation in flow cytometry, imaging, and microplate applications



Cell proliferation assays selection guide

					
Assays and kits	Click-iT EdU and BrdU Assays	CellTrace and CFSE reagents	CyQUANT Cell Proliferation kits	Resazurin (alamarBlue, PrestoBlue)	CyQUANT M and XTT
Mechanism	New DNA synthesis	Generational analysis by dye dilution	Changes in DNA content quantitation	Changes in cellular metabolism, metabolic indicators	

https://www.thermofisher.com/it/en/home/life-science/cell-analysis/cell-viability-and-regulation/cell-proliferation.html