

Cell Spotting

Let's fight cancer together!

DIDACTIC UNIT



socientize
citizen science projects



INTRODUCTION

This didactic unit provides background information and supporting material on the Cell Spotting experiment. It is specially addressed to Biology students from Secondary School. However, any person with interest in contributing to this application can use it as supporting material to the activity and as a learning resource of the scientific contents approached by the application.

In this unit, we try to connect the goals of our experiment with the goals meant for students to achieve in Biology classes.

The main learning goals for the Cell Spotting experiment are:

- To observe cell images obtained with the fluorescence microscope.
- To sketch and legend the main components of the cell.
- To understand the importance of the apoptosis in the growth and cellular renovation.
- To understand the apoptosis and necrosis process.
- To know the principal morphological characteristics of the two types of cell death.
- To recognize the processes of the apoptosis and necrosis in tumour cells through images obtained by fluorescence microscopy.
- To approach students to the techniques used in apoptosis-inducing drug delivery research in tumour cells.
- To introduce the students in advanced research.
- To foster students' participation in cellular biology research.
- To increase academic performance with the work developed by students.

This didactic proposal is structured in the following sections:

- | | |
|---------------------------------------|-------------------------------|
| 1. Introduction | 5.4 Nucleus Shape |
| 2. Before starting... | 5.5 Mitochondria Shape |
| 3. The Cell Spotting Experiment | 5.6 Mitochondria Distribution |
| 4. Cell Death: Apoptosis and Necrosis | 5.7 Cell Mobility |
| 5. To Analyse Cells | 5.8 Cell Shape Changes |
| 5.1 Alive Cells | 5.9 Remarks |
| 5.2 Cell Shape | 5.10 References |
| 5.3 Cell Content Release | |

This didactic unit was not conceived to replace the specific material of the Biology subject. This unit can be used as an additional resource to enrich the lesson plans.

In this unit you will find several symbols, which will guide you through different tasks:



Task. When you see this symbol you will find a task in the didactic unit.



Question. When you see this symbol you will find a question for you to answer in the didactic unit.



Observation. When you see this symbol you will find instructions to perform a step in the Cell Spotting experiment.



Note. When you see this symbol you will find important information to be considered during a certain step.

BEFORE STARTING...

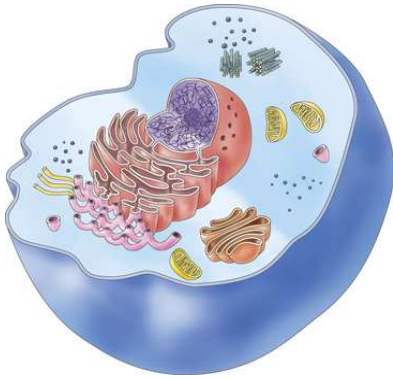


Fig. 1 Representation of an animal cell.

All living beings are made out of cells. There are beings composed by a single cell, like bacteria and protozoa, and others, like plants and animals, with thousands of cells. These multicellular organisms present a high diversity of cells, specialized in certain functions. It is the complex and extraordinary coordination which occurs at cellular level that ensures the internal balance of the organisms.

The human being contains around 200 cell types. These types result from only one cell, the zygote. This particular cell, a product of the fecundation of an oocyte by a sperm cell, begins to divide forming an aggregate of many identical cells that successively divide, giving origin to more specialized cells with certain functions and assuming typical shapes.

In spite of completely different in shape and function, the different kinds of cell types, like neurons and muscle cells, present common fundamental structures. Each cell can be seen as a complex living being that contains small organs, known as organelles, such as the mitochondria or the endoplasmic reticulum. The cell nucleus can be considered as the brain of the cell, being responsible for the management of all cellular activity. Inside the nucleus we can find all the information required to build a new and identical living being. It is like if inside each cell there is a book that describes all the information related to the organism and that the cell consults all the time, to coordinate its activity according to its function. Thus, in spite of each cell being different, the information contained in the nucleus of each cell type is exactly the same, it is the way of being used that is different.

This information is codified in the well-known DNA molecule. In this molecule of double helix, the genetic information is coded in specific segments - the genes. The reading of the genes leads to the synthesis of proteins, essential molecules of life. These molecules have a leading role in cellular activities being present in almost all cellular structures. There are several types of proteins, which means that they also have different functions. Proteins ensure the transport of substances, give support and structure to the cell, catalyze chemical reactions, transcript DNA, and ensure the correct cellular division among others. Proteins are authentic specialized microscopic machines, crucial for the cell and the organism management.

The genetic information is organized in a sequence of specific molecules identified by the letters A, T, C and G - the nucleotides. It is the diversified combination of these molecules that gives origin to different proteins, which with different molecular conformations assume different functions. When, for any reason, the combination of nucleotides changes, the reading of the genes leads to the expression of a protein different from the intended or to no expression at all. These changes, named genetic mutations, can be beneficial or prejudicial to the organism.

Cancer, originated by the uncontrolled division of a specific cell type, is an example of a disease caused by a genetic mutation. By default, when a cell recognizes irreparable errors in the division process, she commits suicide (apoptosis). However, when this regulation system breaks down due to a genetic mutation, the cell divides with no control, threatening the internal balance of the organism.

One of the current approaches to fight cancer consists on the identification of small chemical compounds, possible drugs, that selectively eliminate tumor cells, assaying thousands or even millions of candidates using robotic systems. In these studies, the observation and analysis of the different cell organelles labeled with fluorescent molecules of different colours allows determining the cell response to each chemical compound.

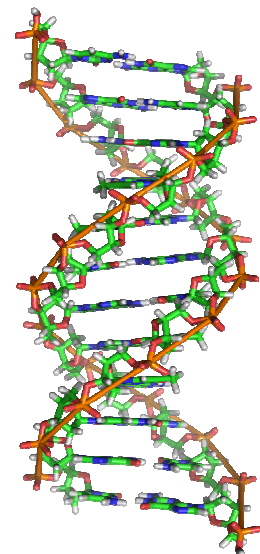


Fig. 2 Representation of the DNA.

THE CELL SPOTTING EXPERIMENT

What is being done

This application was developed to help the researcher José Carrodegua Villar (BIFI, University of Zaragoza) in his investigation about apoptosis, a type of programmed cell death.

One of the main purposes of the investigation is to find chemicals that induce apoptosis in tumour cells.

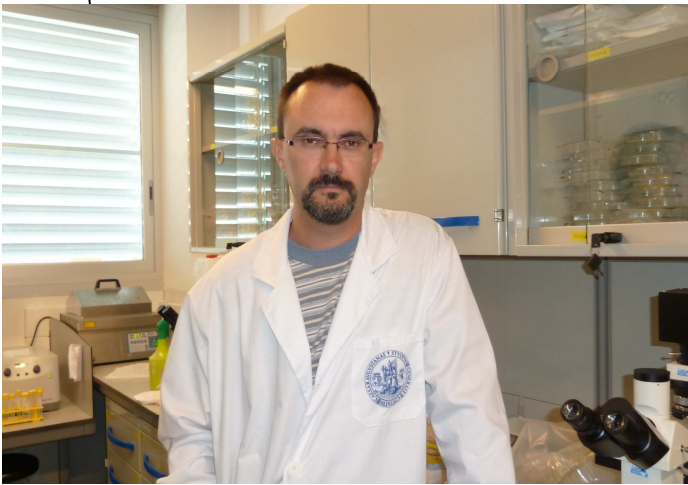


Fig. 3 José Carrodegua Villar

I am especially grateful to all the volunteers who participate in this project. Their work will surely help us progress in the fight against cancer, even if we can only make a very small contribution. I hope volunteers feel like real scientists doing this job and I also hope that this makes them feel science closer and eventually help them move in the near future towards a career in science.

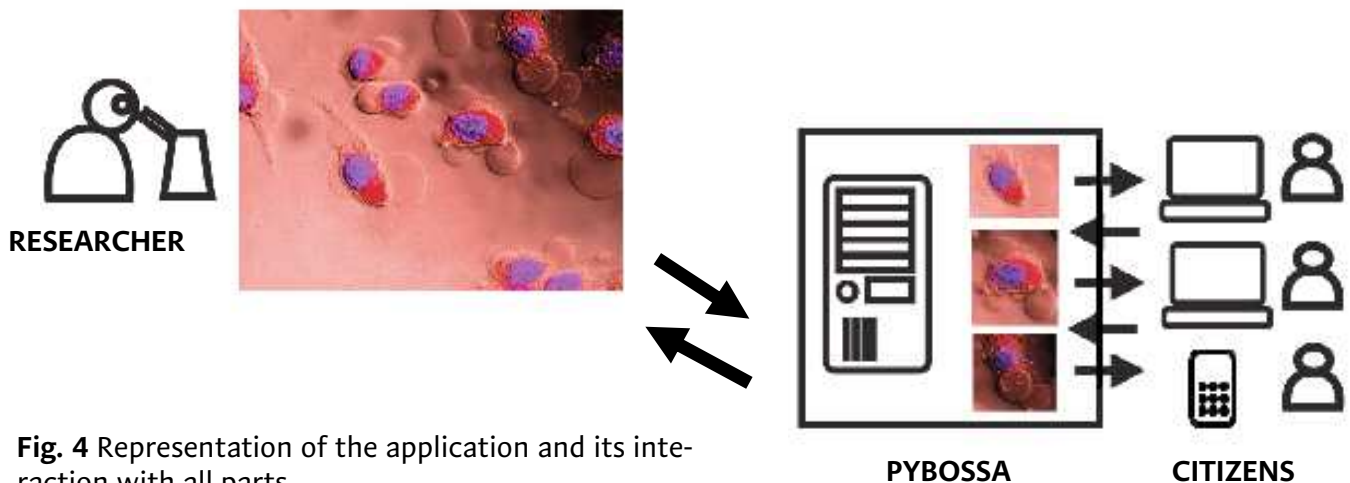


Fig. 4 Representation of the application and its interaction with all parts.

■ How it is being done

José is testing several small chemical compounds in HeLa cells cultured *in vitro*. To determine their effect, José uses an automatic system of microscopy that, at each 30 minutes, takes pictures of the cultures constructing a photographic record of what happens to cells over time. In just one day, José gets more than 4000 images! Thus, the analysis of these images requires a lot of time and, consequently, a lot of money, which delays the development of this important investigation.

You can help!

HeLa Cells

The name has origin in the name of their donor, Henrietta Lacks. This patient had cervical cancer, also known as cancer of the cervix uteri.

■ How you can help

It's simple! All images obtained by José in his lab are sent to the PyBossa platform. In turn, PyBossa distribute these images to citizen scientists, which will analyse them using the Cells Spotting experiment. The generated data is automatically sent to PyBossa at the end of each task, which will send it to José. By answering to basic questions like "Is these cell rounded or elongated?" or "Is this nucleus colour homogeneous or mottled?", you will help José to know in every moment what is happening in each cell culture!

To help José just go to the Socientize website (<http://www.socientize.eu/>), click in the Cell Spotting experiment and **start contributing!**

CELL DEATH: APOPTOSIS AND NECROSIS

Cells have the extraordinary ability of division. This way they ensure the growth of the organisms, cells renovation and the reproduction of all living beings.

As an organism ages, cells lose the ability to divide becoming more liable to errors in the process. When irreversible errors occur, the cell triggers a self-destructing mechanism, named apoptosis. Apoptosis is an extremely important mechanism to the prevention of genetic diseases, like cancer.

However, besides its active participation in the regulation of the cellular division, apoptosis has other functions in the organism. For example, during the human embryogenesis, the cells that occupy the interdigital gaps of hands and feet are destroyed by apoptosis to create fingers. Another example is the proliferation of the mammary gland cells to produce maternal milk. During pregnancy, once breast-feeding is over, the surplus cells activate their apoptotic program and reduce the size of the glands.

Group Work: Search in books or the Internet other examples where apoptosis takes place and prepare a short presentation to your class.

Besides apoptosis, there is another type of cell death with pathologic nature, called necrosis. Necrosis can be caused by microorganisms, virus or chemical agents, and, unlike apoptosis, the cell does not control it. This type of cell death induces lesions at the tissue and organs level triggering an inflammatory response that is not observed in the apoptotic process.

Characterization of the Apoptosis and Necrosis Process

Apoptosis and necrosis are two types of cell death biochemical and morphologically distinct from one another. Therefore, it is very easy to determine through the observation with the microscope the type of cell death suffered by a cell.

Observe the figure 5 and check the main characteristics of apoptosis and necrosis.

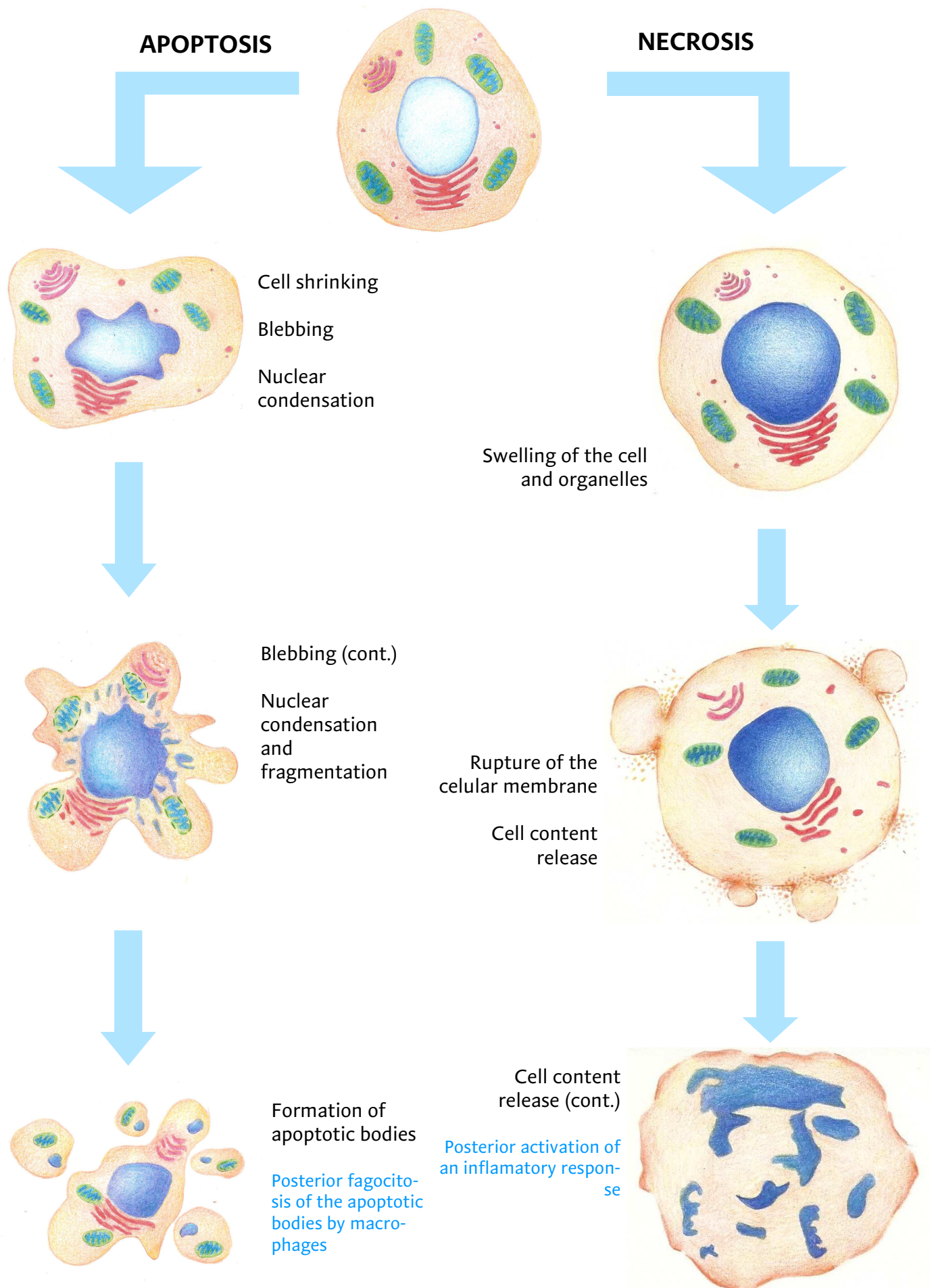


Fig. 5 Representation of cell death: apoptosis and necrosis.

TO ANALYSE CELLS

The images you are about to analyse result from the combination of three different images obtained by fluorescence microscopy. The cell cultures were coloured with fluorochromes, fluorescence colourants that at the microscope reveal the colour blue for the nucleus (Hoechst 33342) and the colour green for mitochondria (Mitotracker) in different transmission channels. The overlay of these images results in a composite image that exposes the cells coloured with blue and green in a bright grey field.



Based on the image below, choose one or two cells to sketch in the square below and indicate the cellular membrane, the nucleus and the mitochondria.

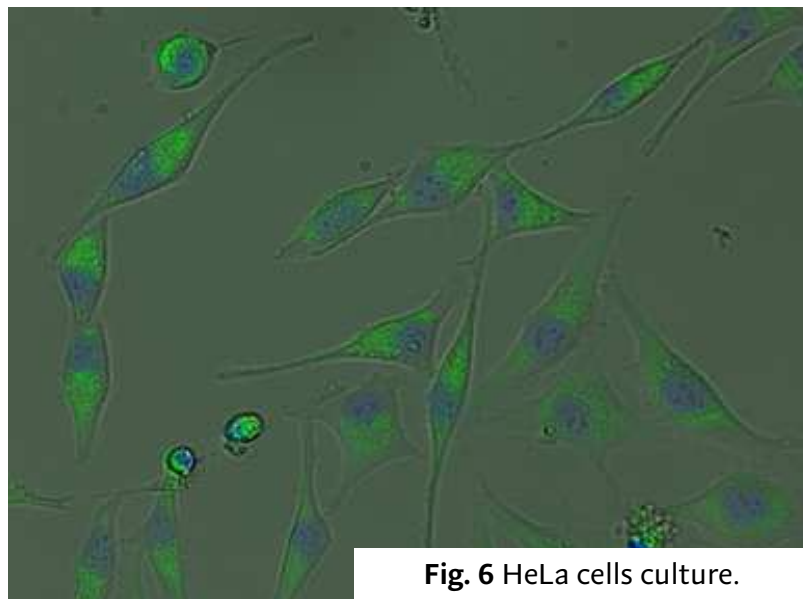


Fig. 6 HeLa cells culture.

Fig. 7 _____

ALIVE CELLS

As you can see in the images below, live cells show different characteristics from dead cells.



Spot the number of live and dead cells.

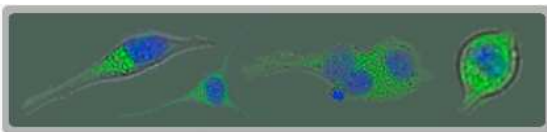


Fig. 8 Live HeLa cells.

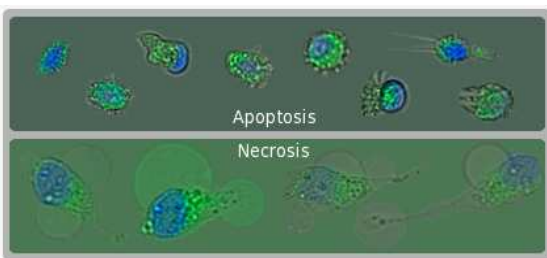


Fig. 9 HeLa cells in apoptosis and necrosis.



This total number of cells will also stay the same in the subsequent steps, as you are always observing the same set of cells. For instance, if you count 35 cells in this first image, then in the rest of the observations you will always have to sum up at 35.

CELL CONTENT RELEASE

One of the symptoms of cell death is content release to the extracellular space. This release is easily detected by the presence of bubbles around the cell.



Spot the cells that released their content and those which did not.

Cell death by necrosis triggers an inflammatory response that is not verified in apoptosis. Why?



A:

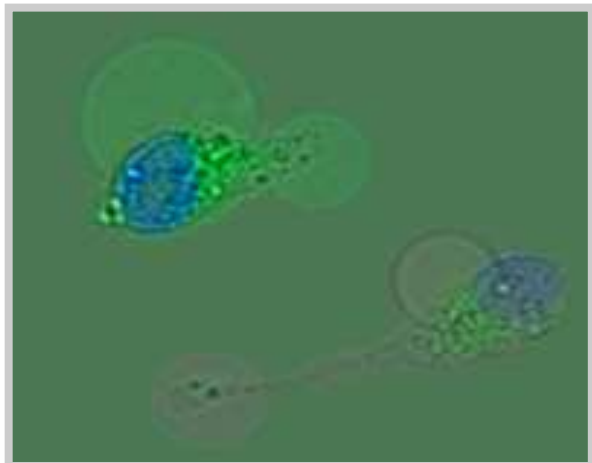


Fig. 10 HeLa cells with content release.

MITHOCHONDRIA DISTRIBUTION

Mitochondria have essential functions in the life and death of cells. HeLa cells, among others, don't show isolated mitochondria like it is represented in textbooks. They show a dynamic and very elongated network of mitochondria normally distributed randomly and evenly in the cytoplasm. During apoptosis, they tend to aggregate around the nucleus in several clusters. Sometimes all mitochondria in a cell aggregate into a clump next to the nucleus with a similar size, suggesting that apart from a blue nucleus, there is also a green one.



Spot the cells that present a scattered and clustered

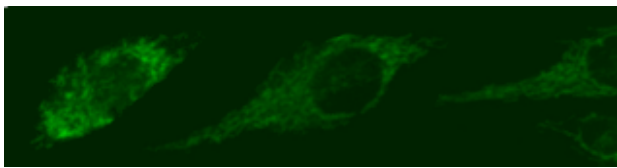


Fig. 11 Scattered mitochondria.

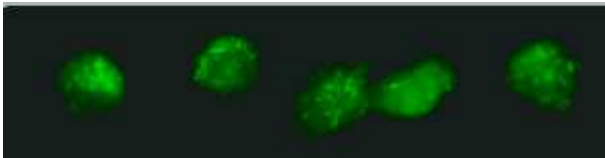


Fig. 12 Clustered mitochondria.

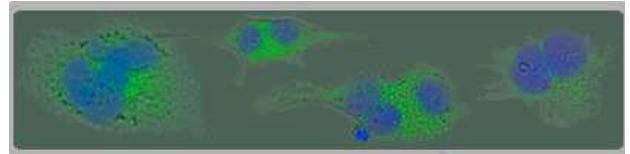
REMARKS

The small chemical compounds in study might have other effects in the cell besides inducing apoptosis. Thus, it is important to record any atypical cell behaviour or change that you find in the tumour cultures. For instance, multinucleated cells suggest the interference of a chemical compound in a certain step of the cellular division process.



Spot the multinucleated cells and any other atypical aspect of the culture that you consider relevant.

Fig. 13 Multinucleated HeLa cells.



Here you have some examples of what can be considered atypical in the cells. Besides these, you are free to name any other aspects that you consider worth of record.

- Multinucleated cells;
- Abnormally large or small cells;
- Abnormally large or small nuclei;
- Cells do not move at all;
- Granularity (little spots) inside the cell;
- Very mobile cells;
- Cell cluster together;
- Cell with aberrant divisions;

CELL SHAPE

Through the shape of a cell is possible to determine the cell status. HeLa cells can be elongated, star-shaped or rounded.



Count the number of elongated (___), star-shaped (___) and rounded cells (___).

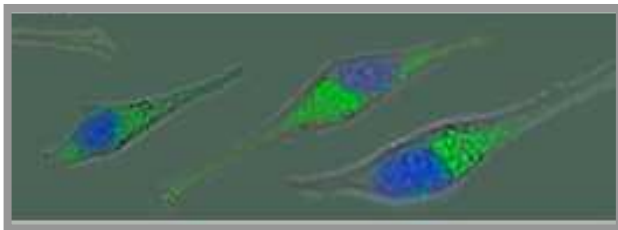


Fig. 14 Elongated HeLa cells.

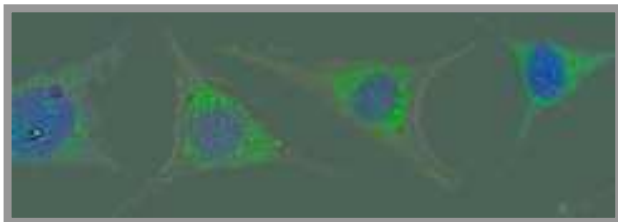


Fig. 15 Star-shaped HeLa cells.

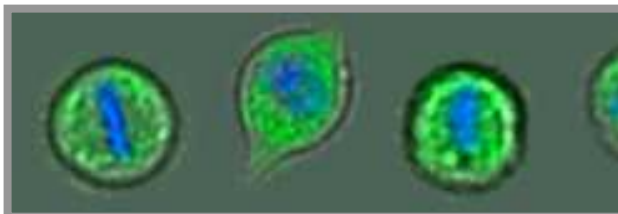


Fig. 16 Rounded HeLa cells.

Which shape(s) might indicate cell death? Why?



A: _____

KNOW MORE ABOUT HELA CELLS AND CELL DEATH!

NUCLEUS SHAPE

Cell death can also be verified at the nucleus level. In the case of apoptosis, the nucleus gets condensed and fragmented.



Count the cells with a rounded (___), elongated (___), bean-shaped (___), and condensed and/or fragmented nucleus shape (___).

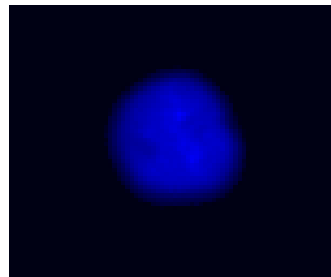


Fig. 17 Rounded nucleus.

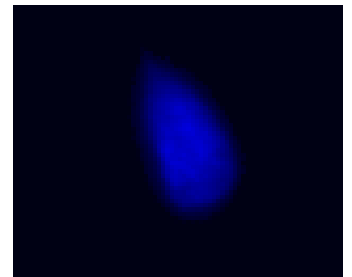


Fig. 18 Elongated nucleus.

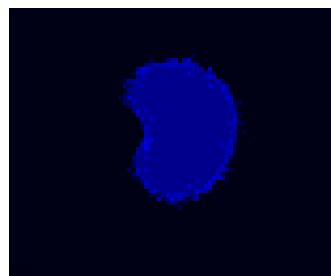


Fig. 19 Bean-shaped nucleus.

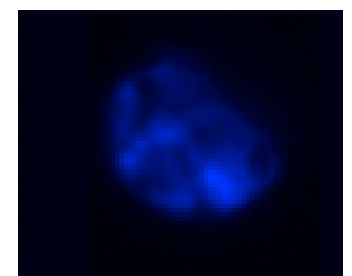


Fig. 20 Condensed and fragmented nucleus.

Why is a condensed nucleus brighter than a rounded nucleus?



A: _____

CELL MOBILITY

Cell mobility is an indicator of the cell's health. A cell with mobility has the ability to move around the culture. A healthy cell normally explores its environment. In the case of a cell in apoptosis, it usually becomes static. Thus, to determine the cell mobility you focus on the movement of the nucleus.



Watch the video of the cell culture and see if any cell is moving!

CELL SHAPE CHANGES

A cell that changes its shape normally corresponds to a healthy cell, with an ability to explore the environment. This observation it is intended to identify those cells that change their shape significantly. To do so, consider cells that present a significant movement of the membrane but keep the nucleus in the same spot.



Watch the video of the cell culture and see if any cell is changing its form!

THE PROJECT

Citizen Science is an innovative concept that intends to establish a link between Society and Science. An effective way to get Society close to Science is by allowing to all citizens their participation in scientific research and experiments. This participation can have many forms and it represents an important contribution for the advance of Science.

Funded by the European Commission, SOCIENTIZE (www.socientize.eu) will coordinate all agents involved in the citizen science process, will promote the practice of scientific volunteering and establish the basis to a new open science paradigm.

Didactic Unit Coordination

MUSEU DA CIÊNCIA
UNIVERSIDADE DE COIMBRA



UNIVERSIDADE DE COIMBRA

With the collaboration of



Universidad
Zaragoza

ZENTRUM FÜR SOZIALE INNOVATION
CENTRE FOR SOCIAL INNOVATION



Universidade Federal
de Campina Grande

tecnara
tecnologías de la información, electrónica y telecomunicaciones de Aragón

REFERENCES

WEBSITES

Projeto SOCIENTIZE
<http://www.socientize.eu>

Projeto IBERCIVIS
<http://www.ibercivis.pt>

BIFI (The Institute for Biocomputation and Physics of Complex Systems)
Jose Carrodeguas Villar
Stem cells and Apoptosis
<http://bit.do/josevillar>

Here you can find more information about Jose's investigation and more videos of HeLa cultures.

BioEd Online
Cells
<http://bit.do/bioed>

Here you can find several lessons about prokaryotic and eukaryotic cells, mitosis, meiosis, cell cycle, membranes, organization and substructures.

Learn.Genetics™
Teacher Resources and Lesson Plans
<http://bit.do/learn genetics>

Here you can find several lessons about the cell's interior, cells communication, evolution and you can also see real cell videos and images.

BOOKS

ALBERTS, B., JOHNSON A., LEWIS, J., RAFF, K.R. & WALTER P. (2002). Molecular Biology of the Cell (4th edition). New York: Garland Science.

LODISH, H., BERK, A., ZIPURSKY, S.L., MATSUDAIRA, P., BALTIMORE, D. & DARNELL, J. (2000). Molecular Cell Biology (4th edition). New York: W.H.Freeman.

PICTURES

Fig. 1 Representation of an animal cell.
Mois Moshev
Illustrator
<http://monomon.me>

Fig. 2 Representation of the DNA molecule.
Zephyris
Richard Wheeler at the English language
Wikipedia
<http://tinyurl.com/oksjuql>

Fig. 5 Representation of cell death: apoptosis and necrosis.
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