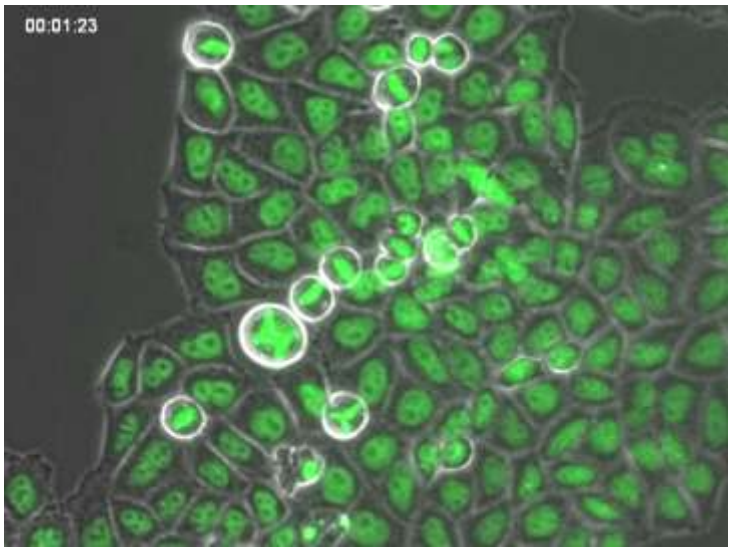
SPARQ-ed

Making Mitosis Movies



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SPARQed is a collaboration between The University of Queensland's Diamantina Institute and The Queensland Government's Department of Education and Training. It exists due to the hard work of the SPARQ-ed Regional Reference Group (Regan Neumann, Associate Professor Nigel McMillan, Associate Professor Brian Gabrielli, Dr Peter Darben, Cheryl Capra, Peter Ellerton, Karen Gosney, Andrew Rhule, Michael Sparks, and Patrick Trussler).

The Mitosis Movies project is based on routine procedures carried out in UQDI's Cell Cycle research group. It was developed by Associate Professor Brian Gabrielli. The experimental program and all supporting materials were adapted for student use by Dr Peter Darben, under the supervision of Associate Professor Brian Gabrielli and Dr Sandrine Roy.

Risk assessments were developed with the assistance of Paul Kristensen, Maria Somodevilla-Torres and Jane Easson.

Many thanks to all in the "Gab Lab" whose patience and support made this happen.

Table of Contents

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Introduction

What is Cell Biology ?

Cells form the basis of all living things. They are the smallest single unit of life, from the simplest bacteria to blue whales and giant redwood trees. Differences in the structure of cells and they way that they carry out their internal mechanisms form the basis of the first major divisions of life, into the three kingdoms of Archaea ("ancient" bacteria), Eubacteria ("modern" bacteria) and Eukaryota (everything else, including us). An understanding of cells is therefore vital in any understanding of life itself.

Cell biology is the study of cells and how they function, from the subcellular processes which keep them functioning, to the way that cells interact with other cells. Whilst molecular biology concentrates largely on the molecules of life (largely the nucleic acids and proteins), cell biology concerns itself with how these molecules are used by the cell to survive, reproduce and carry out normal cell functions.

In biomedical research, cell biology is used to find out more about how cells normally work, and how disturbances in this normal function can result in disease. An understanding of these processes can lead to therapies which work by targeting the abnormal function.

Common Cell Biology Techniques

The following list covers some of the more commonly used cell biology techniques – it is by no means exhaustive.

> ***Cell / Tissue Culture*** – in the same way that bacteria and other simple organisms can be grown in the laboratory outside their normal environment, cells and tissues from more complicated organisms can be cultured as well. The techniques are slightly different, and the culture media are more complex to reflect the complex internal environment inside the host from which the cells are derived, however cell and tissue culture is a powerful tool which provides an almost limitless supply of test material for researchers to use without resorting to using whole organisms. In addition, the controlled conditions in cell and tissue culture allows researchers to carry out experiments with a lower number of variables which may affect the outcome of the test. Cell culture may use cells removed directly from an organism (primary culture), or it may use lines of cultured cancer cells. The benefit of the latter approach is that cancer cells continue to divide, while primary cultures cease dividing after a number of cycles.
>
> ***Microscopy*** – the basic tool of cell biology is microscopy. Recent advances in imaging technology has allowed an unprecedented amount of information to be gleaned from microscopic analysis. Types of microscopic techniques which are used include :
>
> > ***Brightfield*** – traditional microscopy, where cells are illuminated by visible light. Brightfield microscopy gives a general picture of cell function, although that information is not very detailed or specific. As animal cells lack cell walls, brightfield microscopy may use special techniques such as phase contrast to show cellular structures in more detail. Brightfield microscopy allows imaging of live or fixed (dead) cells and tissues)

Electron Microscopy – uses a focused beam of electrons instead of light. Electron microscopy permits a much higher magnification of specimens than light microscopy and is useful in obtaining detailed information about sub-cellular structures. Electron microscopy requires extensive processing and so can only be performed on fixed specimens. Transmission electron microscopy provides a cross section of a specimen, while scanning electron microscopy gives a three-dimensional image of the surface of a specimen.

Fluorescence Microscopy – uses fluorescent materials to indicate structures in a specimen. Fluorescence occurs when light of one wavelength "excites" a material and causes it to emit light of a different wavelength. Most fluorescent materials give off visible light after excitation by ultraviolet light. Structures may be naturally fluorescent (autofluorescence) or they may be labeled with a compound which is fluorescent (eg. DAPI is a dye which binds onto DNA. The DNA and nuclei of cells stained with DAPI emit a blue light under ultraviolet light).

Immunofluorescence – antibodies are proteins made by the immune system which bind onto specific parts of proteins. Antibodies can be raised against any protein in the cell. If these antibodies are attached to a fluorescent tag, the tag will only show up where that antibody attached (ie. where the target protein is found in the cell). Immunofluorescence allows very specific targeting of cellular structures.

RNA Interference – RNA interference uses short sequences of RNA which are complementary to the mRNA which carries to instructions to translate proteins from the DNA to the ribosomes. The interfering RNA binds to the target sequence, preventing it from being translated. As a result, careful selection of interfering RNA can be used to silence a particular gene. This allows researchers to study what role a protein plays in a cell, by observing what happens when that protein is absent.

Timelapse Microscopy – many cellular processes (eg. mitosis) occur over a period of time which is not practical for direct observation. Imaging cells over a period of time (eg. a photograph is taken every 20 minutes for 24 hours) allows us to combine these images in a "movie" which compresses a long time period into a shorter one.

The Project

In this project you will be provided with a culture of human cancer cells. You will treat these cells with anti-cancer drugs which interfere with cell division and then image them over a 24 hour period. You will then combine the images into a "mitosis movie" and analyse the movie to observe the effects of the drugs on the cells.

Getting Started

Before you begin, make sure that you are familiar with the relevant theory behind the techniques we will be performing. This manual contains several appendices which will provide you with this information. Make sure you read this information before proceeding. Additional information about cell and molecular biology can be found at the SPARQ-ed website at : http://www.di.uq.edu.au/sparqed-services#background.

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Theoretical Basis of the Project

The Cell Cycle and Cell Division

The cell cycle represents the normal progression of cells through the division cycle to produce two identical daughter cells. It consists of four discrete stages (See Figure 1):

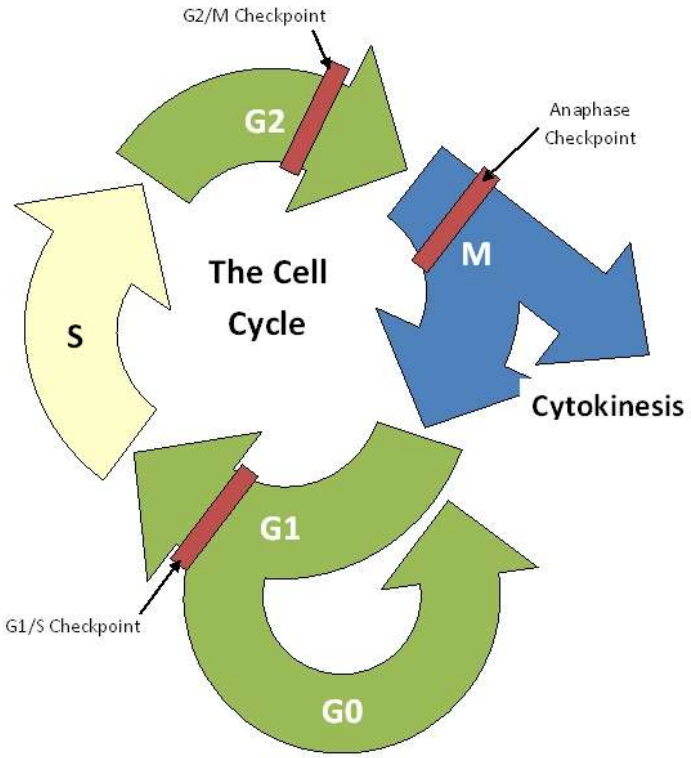


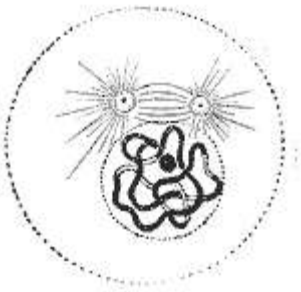
Figure 1 – The Cell Cycle

- G1 (Gap 1) phase occurs just after division. It is where the cell carries out normal metabolism and begins to grow in size and duplicate its organelles. Some cells arrest in G1 phase, staying in a stage called G0 phase and ceasing the cycle of cell division.
- S (Synthesis) phase represents the time where the genomic DNA is duplicated.
- G2 (Gap 2) phase is the time where the cell prepares for division.

Collectively, G1, S and G2 phases are known as Interphase.

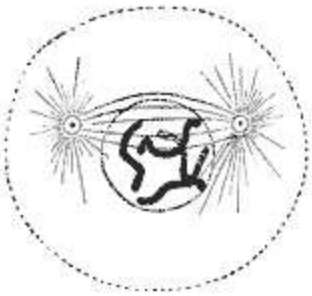
- M (Mitosis) phase is the time when the replicated chromosomes are divided (Prophase, Prometaphase, Metaphase, Anaphase and Telophase (See Figure 2) and the cell splits into two identical daughter cells (Cytokinesis).

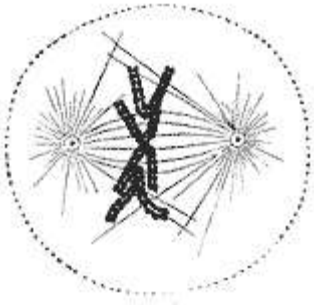
The aim of mitosis is to evenly divide the chromosomes replicated during S phase between the two daughter cells. By the end of S phase, each chromosome consists of a pair of chromatids, joined in the middle by a body called the centromere. During mitosis, it is these chromatids which separate.



Prophase – the centrosomes begin to separate and the chromatin start to condense into discrete chromosomes

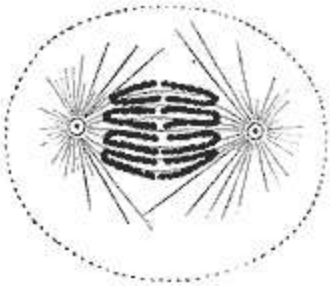
Prometaphase – microtubules extend from the centrosomes to form the mitotic spindle. The chromosomes have condensed and the nuclear membrane has degraded

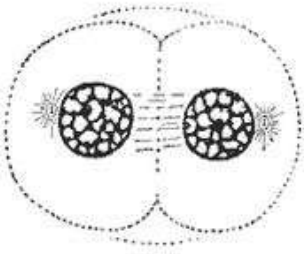




Metaphase – the chromosomes line up along the "metaphase plate" in the middle of the mitotic spindle

Anaphase – the chromatids separate and are drawn to the opposite poles of the mitotic spindle





Telophase – the nuclear membrane reforms around the separate bundles of chromatids, which revert back to non-discrete chromatin. Cytokinesis starts as the membrane pinches off between the two daughter cells

Figure 2 – The Process of Mitosis (Images from Wikimedia Commons)

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The cell assembles several structures which allows the separation of chromatids to occur. The centrosomes are small bodies composed of protein microtubules. Centrosomes are replicated during S phase alongside the DNA. In prophase, the centrosomes migrate to opposite ends of the cell and form the anchor points of the mitotic spindle, a bundle of microtubules which runs between the two centrosomes. In metaphase, the chromosomes attach to the middle of the mitotic spindle at the centromere, via structures called the kinetochores. In anaphase, the kinetochores move towards the centrosomes, dragging the chromatids with them. In cytokinesis, the cell pinches off into two separate daughter cells, each one having a full complement of chromatids.

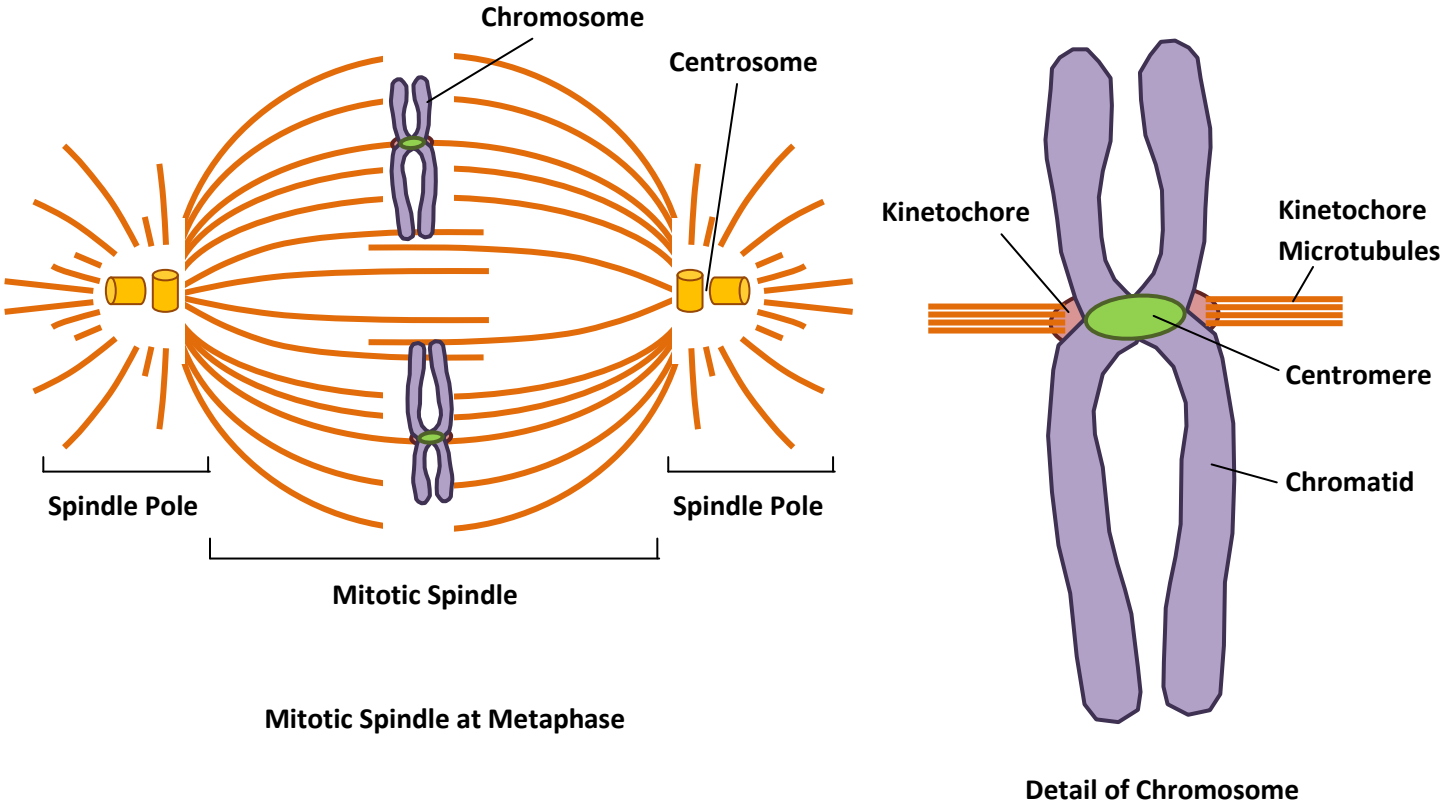

Figure 3 – Chromosomes and the Mitotic Spindle During Mitotis

Normally, progression through the cell cycle is controlled by a series of checkpoints. If a cell cannot meet the conditions needed at each checkpoint, the cycle arrests at that point. This prevents cells being duplicated with significant errors. The major checkpoints occur at the G1/S interface (to ensure that the cell is ready to start DNA duplication), the G2/M interface (to ensure that DNA has been copied without major errors) and at the end of mitosis before the daughter cells divide (to ensure that chromosome separation has occurred correctly). If a cell is found to have problems at any one of these checkpoints (eg. damage to the DNA or an inappropriate number of chromosomes), the cell either repairs the mistake or, if this is not possible, the cell arrests at that point in the cell cycle or even undergoes a process of controlled cell death called apoptosis.

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Cancer and the Cell Cycle

It is important to remember that most cells spend the majority of their time arrested in G0 phase. This is the time where the cells perform their normal functions. Entry into S phase (and ultimately G2 and M phases) only occurs if the cell needs to divide. The majority of body cells do not need to constantly divide – doing so would interfere with their normal function. Therefore most cells stop dividing after a certain number of generations. Replacing these cells is left to specific stem cells which are capable of dividing to produce body cells or more stem cells. Only in parts of the body where there is a high cell turnover (eg. on the body surfaces, in the parts of the bone marrow where blood cells are made, in the hair follicles and parts of the reproductive organs, or after damage has occurred) does one find high rates of cell division.

The collection of diseases called cancer result from a disturbance to this normal order. In cancer, the normal signals which limit cell proliferation are either absent or ignored, and cells spend a larger proportion of time progressing through the cell cycle and dividing. This can occur following mutations (changes in the genetic sequence) or damage to the DNA, particularly when this occurs in the genes which code for proteins which regulate the cell cycle. As a result, studying how the cell cycle is normally regulated is a major step in understanding why cancer develops and how it may be treated.

The cell cycle checkpoints are not physical barriers, but points in the cell cycle where the levels of regulatory proteins build up to a certain level. Each of these regulatory proteins can stimulate or inhibit the expression or function of other regulatory proteins, resulting in a complex feedback system. If something goes wrong with one of these regulatory proteins, it may affect the function of the others, and therefore the whole regulation process.

For example, in the lead-up to the G2/M checkpoint, the cell has just duplicated its DNA and certain cell structures such as the centrosome, which acts as the anchor point for the mitotic spindle. The process of DNA replication is a complex one and, on occasion, substitutions of nucleotide bases may occur. In addition, the cell's DNA may be damaged by chemical or physical factors. For example, ultraviolet light may cause two adjacent thymidine nucleotides to chemically bond together, resulting in a double molecule. Since the polymerase enzymes that replicate DNA use each strand as a template for the formation of new DNA, this "thymine dimer" does not provide the necessary information to the polymerases for them to insert the correct complementary bases (in this case it would be two adenosine nucleotides) and a mutation is generated.

If a checking and correction mechanism was not present, any mistakes in the DNA would be passed on to the daughter cells. At the G2/M checkpoint, the cell checks the DNA to ensure that no such mistakes are present. If any are found, enzymes in the cell try to repair the damage. The cell pauses in the cell cycle at this point until the damage is repaired, whereupon the cell is released into mitosis. If the damage cannot be solved, the cell is not permitted to enter mitosis, thus limiting the problems associated with passing on these errors.

Problems arise when the errors occur in the genes involved in this regulatory process. If the proteins whose task it is to check the DNA, repair any damage or prevent the cell from progressing into mitosis until the damaged is repaired are themselves faulty, cells will be admitted into mitosis with the errors still in place and the cycle will continue. One of the hallmarks of cancer is hyperproliferation, where cells continually divide because the regulatory processes have been impaired. Cells which are constantly dividing do not carry out their normal function (eg. in leukaemia, a cancerous transformation of the stem cells which give rise to the

white blood cells, a large number of white blood cells are produced, however these never mature to carry out their normal role of defending the body. Therefore people with leukaemia may be at a higher risk of developing infections).

Some external factors may interfere with the normal functioning of the regulatory process. The human papillomavirus has a pair of oncogenes (genes associated with the development of cancer). When the virus incorporates these into the genome of the host cell and they are expressed, the proteins produced inhibit the regulatory processes and the cells progress through the cell cycle. This suits the purposes of the virus, as it requires actively dividing cells in order to proliferate. However, the loss of cell cycle regulation means that more errors in DNA are allowed to pass and the cells accumulate enough of these mistakes to transform into cancerous cells.

Another characteristic of cancer is aneuploidy, where cells have an inappropriate distribution of chromosomes. This can occur in damaged strands of DNA which fragment during division. Under some circumstances the mechanisms which govern cell division may also be affected. In normal cells, the centrosome is replicated during S phase, and each centrosome migrates to opposite ends of the cell to create the mitotic spindle. When the daughter cells pinch off during cytokinesis, each cell should only have one centrosome. Sometimes an extra centrosome is retained or replicated in the cell, resulting in a mitotic spindle with three or even four poles. This means that when the chromatids are separated during anaphase, they may be pulled in 3 or even four different directions. The daughter cells which result from this sort of division are highly likely to be aneuploid and therefore lack important genes.

Cancer Treatment – Chemotherapy and Radiotherapy

Treating cancer is different to treating other diseases. If the body is being attacked by a foreign organism, we can target therapies which take advantage of how that organism is different to us. For example, some antibiotics like the penicillin family target bacterial cell walls, a structure lacking in our cells. With cancer, the disease agent is one that is derived from our own cells. To effectively treat cancer, we use therapies which target cellular functions that cancer cells do more than our normal body cells do – like constantly pass through the cell cycle.

Radiotherapy is the use of radiation to target cancer cells. It relies on the idea that certain types of ionizing radiation damages DNA. In normal cells this results in damage which either the cells can repair or a relatively low level of cell death, whereas in rapidly dividing cancer cells without functioning regulatory mechanisms and checkpoints, the damage is highly lethal. The principle is therefore to hit as many rapidly dividing cells as hard as possible, with the reasoning that the majority of these will be the cancer cells we want to kill. Unfortunately, the body also has rapidly dividing cells which are not cancerous, and so the side effects of radiotherapy may include symptoms which reflect this - anaemia and low white cell count as blood stem cells are affected, gastrointestinal upset as the cells lining the digestive tract are affected, loss of hair as the cells in the hair follicle are killed. Using radiotherapy therefore involves carefully targeting and control of the dose given to ensure that the number of normal cells lost is minimal.

Chemotherapy is the use of chemical drugs to target cancer cells. While some chemotherapeutic drugs use the same approach as radiotherapy (ie. targeting rapidly dividing cells), other drugs target systems more specific to the cancer cells. For example, a certain subset of breast cancers are stimulated to grow by the oestrogen

hormones. Tamoxifen is a drug that blocks this stimulus by simulating the chemical structure of the oestrogens, thus slowing the progression of the cancer. However, not all oestrogen responsive cells are cancerous, and the drug still has some side effects. More recently, research has turned its attention to developing drugs which specifically target what has gone wrong in the normal regulatory processes of the cell cycle. These new agents may inhibit the expression of factors which promote uncontrolled cell proliferation, or they may stimulate or even mimic the regulatory proteins which limit progression through the cell cycle. The effects of these drugs can be seen by studying what happens to the cells as they divide.

Investigating the Effects of Chemotherapeutic Drugs Using Mitosis Movies

Cancer cell lines are commonly used by cell biologists as models for how cells function. They have the benefit of being "immortal" (ie. they constantly pass through the cell cycle), so fresh cells do not need to be sourced from new tissue. The cancer cell line you will be using are called "HeLa". These cells are derived from a tumour removed from a woman named ***He***nrietta ***La***cks in the early 1950s, and were one of the first widely used cancer cell lines. HeLa cells are robust and serve as an effective model system for both cancer investigations and other cell biology studies. The HeLa cells you will be using have been genetically modified to contain a green fluorescent protein tag on one of its histones. In the nucleus, the DNA wraps around these proteins, so the green fluorescent signal can be used as an indication of where the DNA is in the cell. Under blue light, the nuclei of these cells emit a green fluorescence.

The drugs you will be using cover a range of modes of action, although each of them is effective in slowing cell proliferation.

> ***Taxol*** – derived from an extract of the Yew tree (***Tax****us brevifola*), Taxol has been used as a chemotherapeutic agent for nearly 40 years. Taxol stabilizes the microtubules which form the mitotic spindle. The process of spindle formation is normally a dynamic process, involving the assembly of breakdown of the microtubules. In the presence of Taxol, this process is interrupted and the chromosomes cannot arrange appropriately on the spindle. Cells treated with Taxol tend to arrest at the M Phase checkpoint and often undergo apoptosis before cell division is complete.
>
> ***PLK1 Inhibitor*** – PLK1 (polo-like kinase 1) is an enzyme involved in the formation and maintenance of the mitotic spindle. Overexpression of the gene for PLK1 has been seen in cancerous cells, suggesting that it has a role in tumorigenesis. Conversely, removal of PLK1 from systems encourages cells to arrest in mitosis and enter apoptosis. This drug inhibits the action of PLK1, resulting in increased cell death.
>
> ***SBHA (Suberoyl bishydroxamic acid)*** – is a histone deacetylase inhibitor. These drugs work by promoting the expression of genes which inhibit the processes which lead to a loss of cell cycle regulation. Cells treated with histone deacetylase inhibitors undergo an aberrant mitosis and cytokinesis then tend to undergo apoptosis
>
> ***ICRF-193*** - This drug inhibits the action of topoisomerase II, an enzyme involved in the repair and maintenance of the DNA strand. As errors are found in the DNA, damaged parts are cut out of the DNA strand and the two ends of the strands joined back together – both of these functions are carried out by topoisomerase enzymes. ICRF-193 prevents topoisomerase II from re-joining the DNA strands, resulting in fragmentation of the DNA. This causes the cells to arrest at the G2/M checkpoint and then enter mitosis where they may fail to divide their replicated genomes.

In addition to the four known drugs, you will also investigate the action of a new drug which has a similar mode of action to the others. By observing how this drug affects the cells, you should be able to classify it as being related to one of these drugs.

Experimental Protocol

How to Use this Manual

Throughout this section you will see a series of icons which represent what you should do at each point. These icons are:


Write down a result or perform a calculation.


Prepare a reaction tube.


Incubate your samples.

When you are asked to deliver a set volume, the text will be given a colour representing the colour of the micropipette used:

e.g.	750µL	Use the blue P1000 micropipette (200-1000µL)
	100µL	Use the strong yellow P200 micropipette (20-200µL)
	15µL	Use the pale yellow P20 micropipette (2-20µL)
	2µL	Use the orange P2 micropipette (0.1-2µL)

Part A – Treatment of Cancer Cell Cultures with Chemotherapeutic Drugs

You will be provided with a culture plate containing six wells. Each well contains the HeLa cells we will be using in our study. The cells were added to the wells in the form of a suspension, and, as they sunk to the bottom of the well, they stuck to the plastic and started to divide. They were then incubated until the cells covered a large proportion of the plastic (confluence). In addition to being a place where the cells grow, each well will act as a treatment chamber and "slide" as the microscope we use can view the cells through the bottom of the well. The cells adhere quite strongly to the bottom of the wells. This means that the cell culture liquid can be replaced and the cells can be washed without any loss.

The plate provided contains cell culture media. As eukaryotic cells are more complex than bacterial cells, the medium used to grow them is more complex as well. This medium may consist of :

- Basal Medium – a commercial broth containing salts, sugars, buffers and other nutrients needed by all cell culture lines. These media are often coloured pink due to the presence of a pH indicator. As the cells metabolise, they produce wastes which change the colour of the medium to yellow, which can be used as an indicator of when the medium should be changed.

- Foetal Bovine Serum (FBS, also called Foetal Calf Serum) – the liquid component of clotted blood from a bovine foetus. This provides a complex series of nutrients required by the cultured cells.

- Pen/Strep/Glut – a mixture of the antibiotics penicillin and streptomycin, and the amino acid glutamine. The antibiotics prevent the growth of bacteria which may contaminate the medium and interfere with the growth of the cells. The glutamine supports the growth of rapidly growing cells.

- Sodium Pyruvate – pyruvate is the product of glycolysis and is the feedstock for the citric acid cycle in cellular respiration. It is included as an additional energy source.

- HEPES Buffer (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) – a buffer which is particularly efficient in resisting the changes in pH caused by the release of carbon dioxide by cells

Unfortunately, the cell culture media used to grow these cells is incompatible with the optimal conditions for time-lapse imaging of the cells. Therefore, the cells must be washed and the medium replaced with another before proceeding.

Preparation of Cell Culture Media, Phosphate Buffered Saline and Drug Solutions

To prevent contamination, all of the following procedures must be carried out in the tissue culture hood.

To a 50ml centrifuge tube, add the following components :

Culture Medium

Hams F12 Basal Medium	Serum Supreme
45mL	5mL

You are provided with phosphate buffered saline (PBS) at a concentration which is ten times (10X) more concentrated than what is needed – this is known as a stock solution. Stock solutions are made at a higher concentration and then diluted as necessary to provide working solutions (1X). The use of stock solutions saves us the bother of having to make up new solutions every time they are needed.

You need to make your working solution of PBS by diluting it appropriately in water. You are provided with a 100mL sterile "Baxter" water bottle for this purpose. The process for working out what volume to add to the Baxter water is as follows :

- Total volume = 100mL.
- 1/10 of 100mL = 100 ÷ 10 = ____ mL.

∴ volume of 10X stock needed is ____ mL.

- Remove this volume from the Baxter water bottle.
- Add this volume of 10X PBS stock to the Baxter water bottle.

The Taxol is also provided as a 10X stock and will need to be diluted 1 in 10, however the amount needed is significantly less than the PBS.

- for our purposes, 10µL should be sufficient
- Total volume = 10µL.

1/10 of 10µL = 10 ÷ 10 = _____ µL.

∴ volume of 10X stock needed is _____ µL.

∴ volume of culture medium needed is 10 - _____ µL = _____ µL

Combine the calculated volumes of Taxol Stock and culture media in a labelled Eppendorf tube.

Treatment of Cells

In the tissue culture hood :

- Remove the cell culture media from each of the wells using the vacuum line

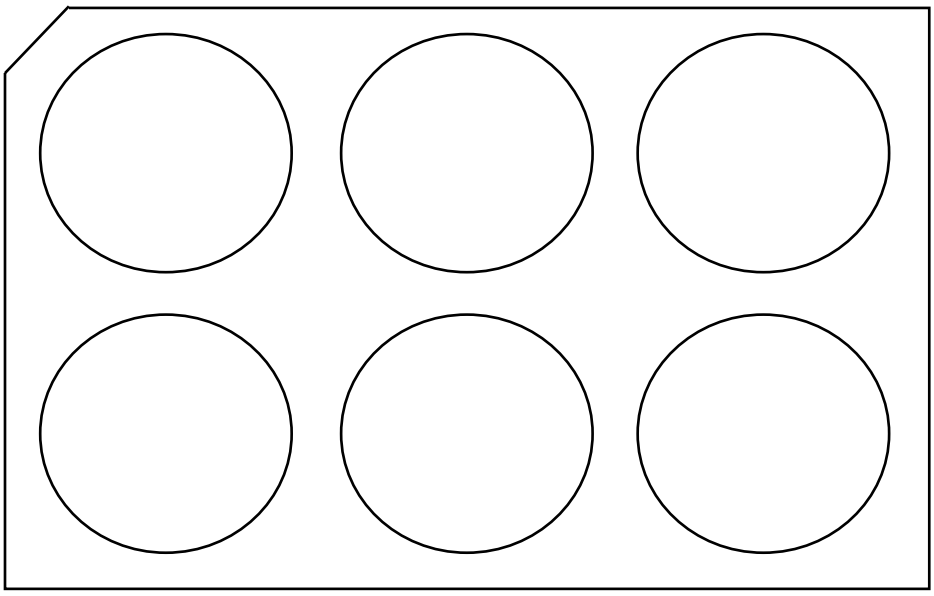
Add 3mL of 1X PBS to each of the wells

- Remove the PBS from each well using the vacuum line

Add 3mL of the prepared F12/Serum Supreme culture medium to each of the wells

To each of the wells, add 3µL of the drug to be investigated. Leave one well untreated – this will be your negative control which should show how the cells divide in the absence of the drug. You will compare the results of your drug to this control when analyzing your results.

- Place the culture plate in the 37°C CO_2 incubator
- In the diagram below, record which treatment was performed in each well.



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Part B – Microscopy and Analysis of Results

Setting Up and Running the Microscope

Your plate will be taken to the live cell imager located in the Ground Floor laboratories of UQDI. This consists of a microscope with a miniature incubator built into the stage. This incubator is the same as the larger ones which we use to grow the cells, maintaining them at a constant temperature of 37°C in an atmosphere containing 5% CO_2.

This is an inverted microscope, meaning that instead of the lenses being placed above the specimen as in a normal microscope, the lenses are located beneath, closer to the bottom surface of the wells in the plate where the cells are found. Light is shone down through the culture plate and the lenses capture images of the cells stuck to the bottom.

When we place the plate on the microscope, we select five suitable fields in each well, programming these into the automated stage. The microscope will return precisely to each of these fields every time it takes a photograph, every 20 minutes.

Each time the microscope images a field, it takes two photographs. One image shows the cells under phase contrast a form of brightfield microscope that accentuates the contrast (animal cells lack cell walls and are difficult to see without staining). The second photograph is taken under blue light and shows the green fluorescence signal generated by the GFP tagged histones. Note that while the light given off by the nuclei in this image is green, the strength of the signal is quite weak. A black and white camera is used to image both the brightfield and fluorescent fields as these are more sensitive to dim light than colour camera. The microscope's software gives a false green colour to the fluorescent image and then combines the two images together (see Figure 4).

The microscope will take two images of each field, five fields to a well for all six wells, every 20 minutes over a period of 24 hours. When it is finished, the images will be processed and combined then downloaded and brought to you to make into a movie.

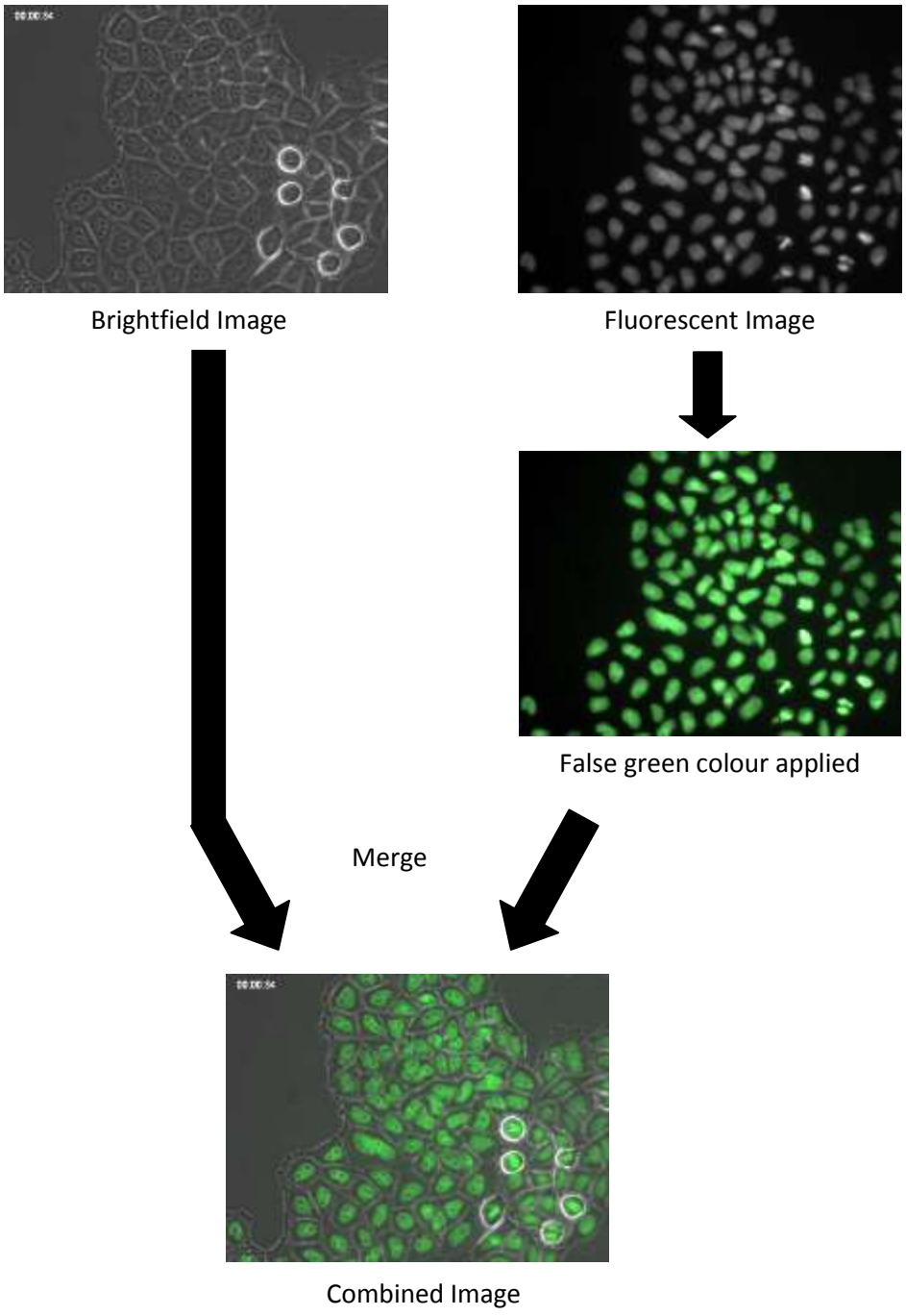

Figure 4 – Process of Creating Combined Images on the Live Cell Imager

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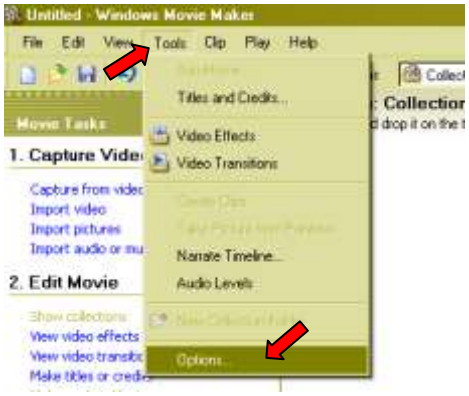
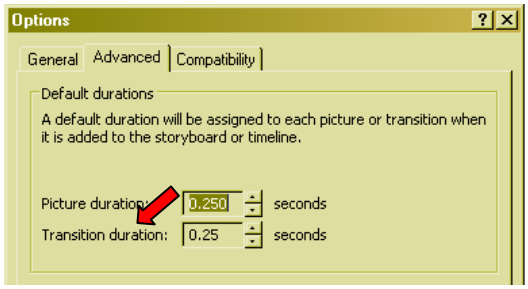
Making the Movie

You will be provided with all of the combined images from all five fields in each well. It is then a relatively simple task to join all of these images together to create a movie. In the example below, we will be using Windows Movie Maker to do this.

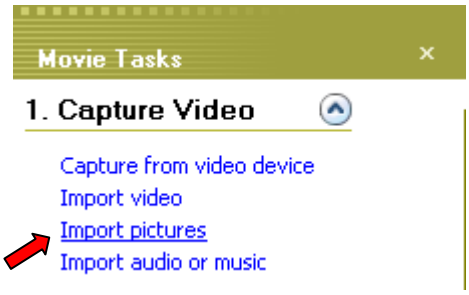
- Open Windows Movie Maker and click "Tools" > "Options"

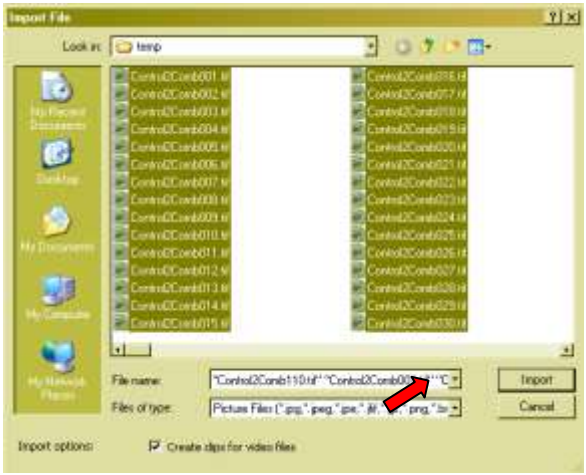




- Under the "Advanced" tab, set the Picture duration and Transition duration both to 0.25 seconds. Click "OK"

- From the "Movie Tasks" menu on the left hand side, select "Import Pictures" and find the directory where your images are stored.





- Select all of the images and click "Import". The images should now appear in the "Collections" pane in the middle of the screen.

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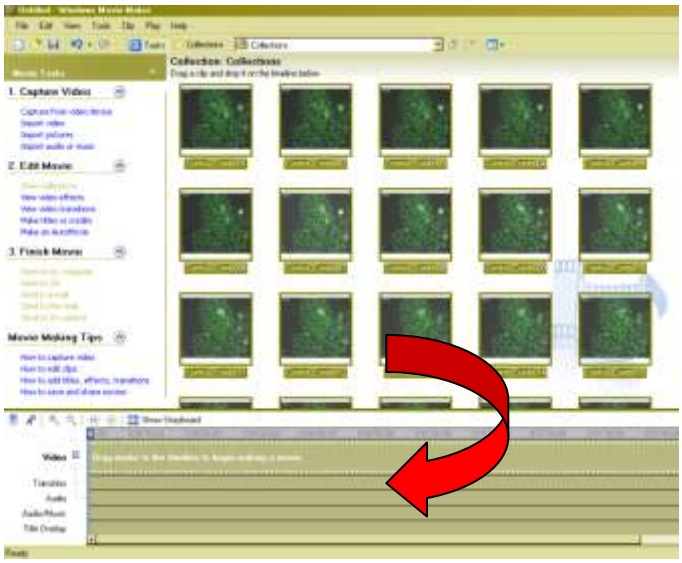
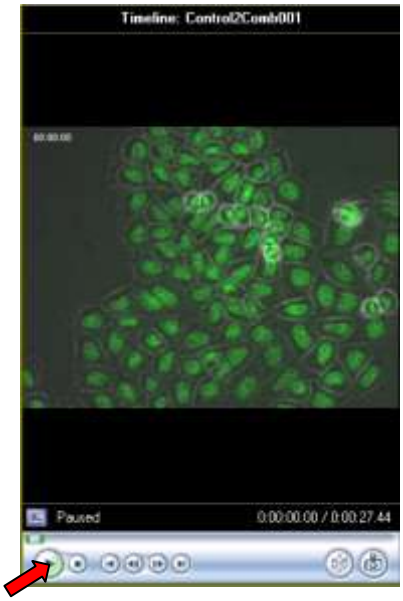
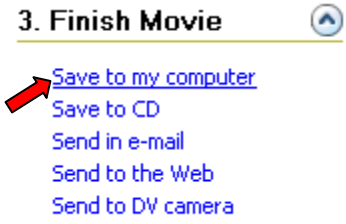
- Select all of the images from the selection pane and drag them down into the storyboard along the bottom of the screen.





- Preview the movie by clicking "Play" (▸) on the preview player on the right hand side of the screen. Edit the movie if necessary

- Make the movie by selecting "Finish Movie" > "Save to My Computer" from the menu on the left hand side of the screen and following the prompts.



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Analysis of Results

Your aim in this analysis is to observe any changes between the patterns of cell division between the untreated control cells and each of drug treated cells. Examples of difference which may be encountered include :

- Delays in entry into or departure from mitosis
- Unusual patterns of apoptosis
- Abnormalities during mitosis (eg. tri- or quadripolar spindles, failure of cytokinesis)

The easiest way to do this is to take a tally of cells exhibiting the trait you are looking for in each image and graph this as a value over time. You could do this analysis for every image individually, however in the interest of saving time (and eyestrain), you can use the movie you have made and select time points through it.

What to Look For

Figure 5 shows series of images which follow a cell as it passes through mitosis

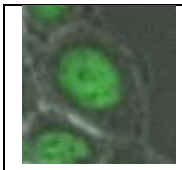 a) A typical interphase cell. Note that the fluorescent signal is dim and the outer edges of the cell are indistinct as it lies flat against the plastic.	 b) As the cell enters mitosis, it starts to "round up". Its outer surface can be seen as a bright halo around the cell and the chromatin is less regular.
 c) In prophase and prometaphase, the chromatin starts to condense, resulting in a stronger fluorescent signal.	 d) In metaphase, the chromosomes form a characteristic bright green band across the middle of the cell (the metaphase plate)
 e) Following anaphase where the chromatids separate, the cell enters telophase where the chromosomes unwind and start to become less distinct. Note that cytokinesis is almost complete, with two daughter cells and that this process is highly symmetrical.	 f) The two daughter cells in late telophase passing into interphase. Note that the cell boundaries are becoming less distinct as they flatten back onto the surface.

Figure 5 – Progression of a Cell Through Mitosis

From these images, it is relatively easy to see when a cell enters mitosis (it rounds up, forming the bright halo around it), when mitosis is completed by the appearance of two equivalent anaphase chromatin bars and when it completes cytokinesis (the bright membrane pinches off to form two daughter cells).

Apoptosis is a process of controlled cell death. When cells are damaged and they break down in an uncontrolled fashion, the cell contents are released into the surrounding tissue and these may affected other cells (sometimes triggering damage which causes more necrosis). In apoptosis, cell components are packaged up into discrete membrane-bound parcels which are then disposed of by immune cells without causing damage. Apoptosis is the method the body uses to dispose of cells which are damaged or otherwise faulty, or

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which are no longer needed (eg. removing the webbing between the fingers and toes during normal foetal development).

Apoptotic cells show nuclear staining as bright as mitotic cells but present as small discrete bundles (representing the nucleus being parceled up into membrane-bound bodies) and a distinct irregular, "bubbly" appearance to the cytoplasm and outer membrane (see Figure 6).

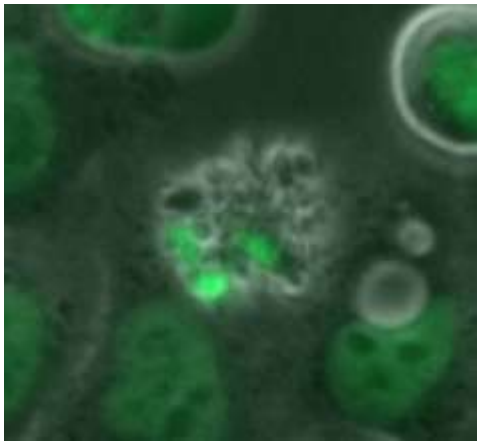

Figure 6 – Apoptotic Cell

You will be recording the following information about each cell on the field :

- Frame at which the cell enters mitosis (ie. when it rounds up). This is a cumulative score – once a cell is counted it is part of the count permanently.
- Frame at which the cell leaves mitosis (ie. when mitosis is complete by the appearance of two anaphase chromosome bars). This is also a cumulative score.
- Time in mitosis (the first value above subtracted from the second)
- Does cytokinesis occur and is it normal (ie. giving two equivalent daughter cells)
- Does apoptosis occur ? If so, when (frame number) and at what stage (before mitosis, during mitosis, after cytokinesis, etc)

Performing the Analysis

- Progress through the movie frame by frame (you can do this using the movie, or you can use the raw images for this)
- You will follow each cell as it enters mitosis
- Record this information in a table or spreadsheet similar to the one overleaf.

Analysing the Results

The simplest analysis to perform is to graph the cumulative percentage of cells in mitosis against time (frame). In untreated control cells, this should yield a roughly straight line (see Figure 7).

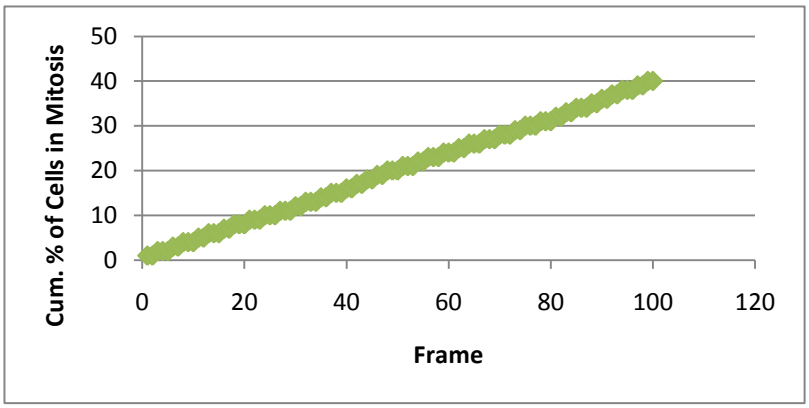

Figure 7 – Progression of Control Cells Through Mitosis

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Cell	Frame Enters Mitosis	Frame Leaves Mitosis	Time Spent in Mitosis	Cytokinesis	Apoptosis

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Any changes to mitosis will then be seen as a deviation from this relationship. Figure 8 shows a treatment which results in a delay in entry into mitosis.

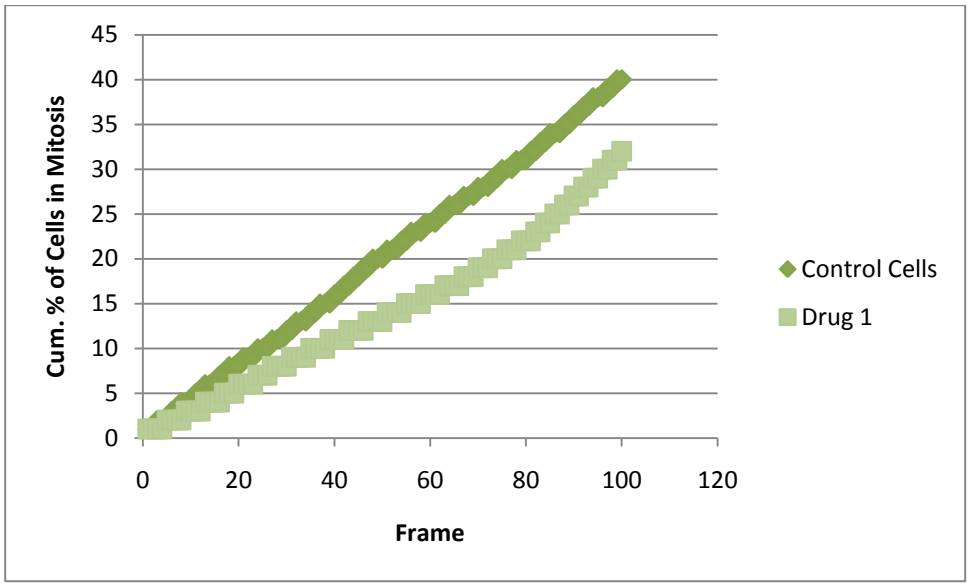

Figure 8 – Comparison of Untreated Control Cells to Drug Treated Cells

A similar time series can be plotted for the cumulative number of cells which undergo apoptosis.

Delays in the time spent in mitosis can also be presented to compare the effects of different drugs. In the scatter diagram below (Figure 9), the time spent in mitosis has been plotted for control cells, as compared to two other drugs.

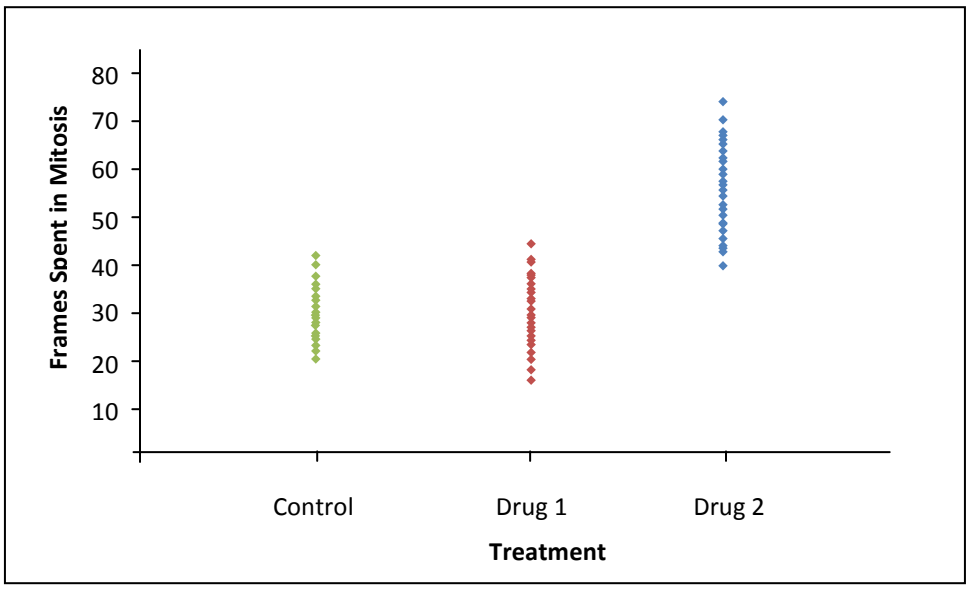

Figure 9 – Comparison of Time Spent in Mitosis for Three Drug Treatments

Conclusions

From a detailed analysis of the results, you should be able to answer the following questions :

- What differences are there between the progression of cells treated with each of the drugs tested and that of the untreated control cells ?

- Can you match these effects with the mode of action of each of these drugs described earlier ?

- Are there any drugs whose effects are similar to the unknown experimental drug ? If so, what category of drugs do you think this new drug belongs to ?

- Did you notice anything else occurring in the treated cells that was different to the control cells ? What do you think might have caused this ?

Further Investigations

- What is the average time for these cells to complete mitosis ?

- We only provided you with minimal information about the modes of action of each of the drugs used. Research more information on each of these drugs (topics could include : structure of the drug, other drugs with similar activities, how extensively the drugs are used, what cancers they are used to treat). You could also research other chemotherapeutic drugs and predict what effect they would have on the cell cycle.

- Using images from your raw data, construct a poster showing the different stages of the cell cycle and mitosis.

- Through careful cropping of the control cell raw images, construct a movie showing just one cell passing through mitosis that could be shown to younger science students at your school. Use the editing function on Movie Maker to insert captions, a soundtrack and even a commentary.

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Appendix A : DNA

- Deoxyribonucleic acid (DNA) is a large molecule which stores the genetic information in organisms. It is composed of two strands, arranged in a double helix form. Each strand is composed of a chain of molecules called ***nucleotides***, composed of a phosphate group, a five carbon sugar (pentose) called deoxyribose and one of four different nitrogen containing bases.

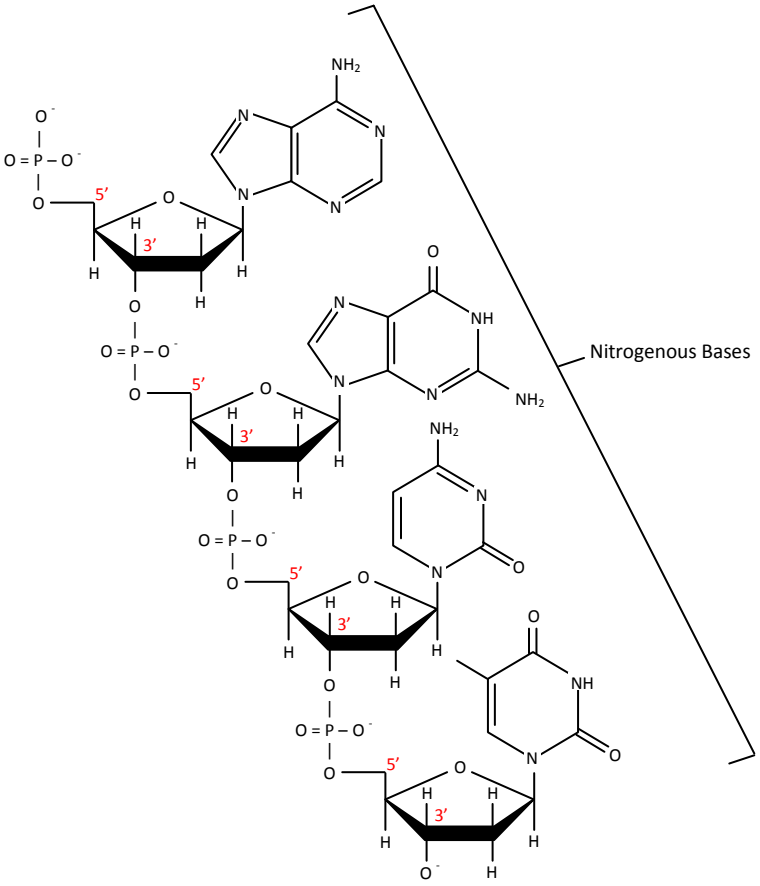

Figure A1 – The Structure of a Single Strand of DNA

- Each nucleotide is connected to the next by way of covalent bonding between the phosphate group of one nucleotide and the third carbon in the deoxyribose ring. This gives the DNA strand a "direction" – from the 5' ("five prime") end to the 3' ("three prime") end. By convention, a DNA sequence is always read from 5' $\rightarrow$ 3' ends.

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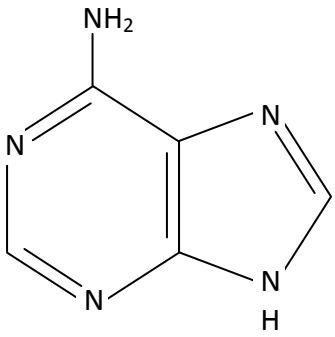
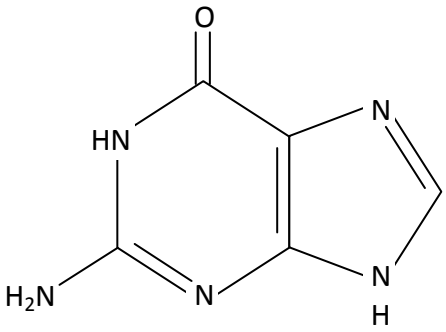
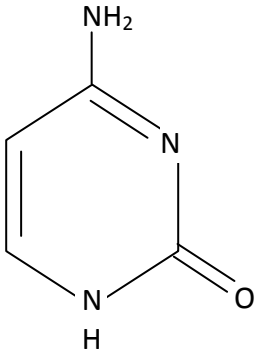
- DNA nucleotides contain one of four different nitrogenous bases:



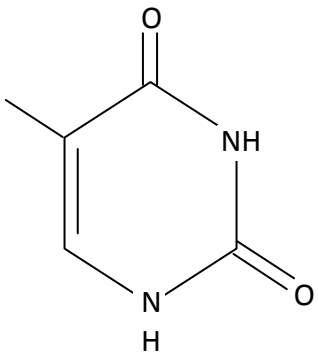
Adenine



Guanine



Cytosine



Thymine

Each of these bases jut off the sugar-phosphate "backbone". If the double helix of the DNA molecule can be thought of as a "twisted ladder", the sugar-phosphate backbones form the "rails", while the nitrogenous bases form the "rungs".

- The two strands of DNA are bound together by hydrogen bonding between the nucleotides. Adenine always binds to thymine and guanine always binds to cytosine. This means that the two strands of DNA are ***complementary***. The complementary nature of DNA is allows it to be copied and for genetic information to be passed on - each strand can act as a template for the construction of its complementary strand.

- The order of bases along a DNA strand is called the ***DNA sequence***. It is the DNA sequence which contains the information needed to create proteins through the processes of transcription and translation.

- Each strand of DNA is ***anti-parallel***. This means that each strand runs in a different direction to the other – as one travels down the DNA duplex, one strand runs from 5' $\rightarrow$ 3', while the other runs 3' $\rightarrow$ 5'.

- An animation of the structure of DNA can be found at:
  http://www.johnkyrk.com/DNAanatomy.html

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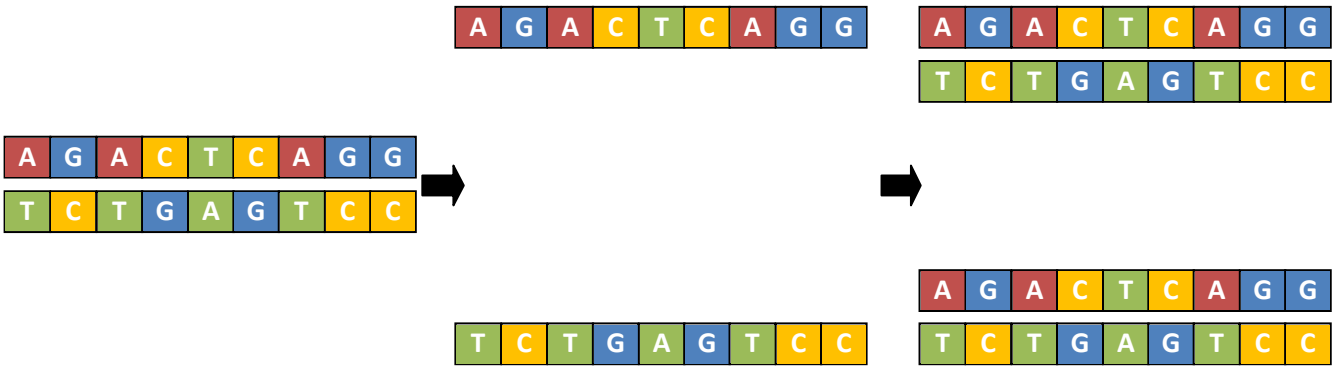
Appendix B : DNA Replication

- The structure of DNA allows it to carry out two vital functions for the cell :
  - Encoding the information need to build and regulate the cell, and
  - Transmission of this information from generation to generation

- In order for the genetic information to be passed on, it must be copied. DNA replication occurs during the S (synthesis) phase of the cell cycle. It only proceeds if the G1 checkpoint is passed, which ensures that the chromosomes have properly segregated during mitosis.

- In simple terms, DNA involves the separation of the two strands of the DNA molecule and the construction of complementary strands for each one, using the A → T, G → C binding rules.



Strands separate

A new strand is built for each, using the original strand as a template

- Because the two new strands of DNA each contain one of the original parental strands, the process of DNA replication is said to be ***semi-conservative*** (ie. half of the new DNA molecule are strands "saved" from the parental molecule).

- Naturally, the process of replication is a more complicated process than simply matching nucleotide bases. Copying DNA involves the interplay of a series of enzymes and regulatory processes, all kept in check by stringent error checking and repair mechanisms.

- DNA replication begins when the enzyme ***helicase*** "unwinds" a small portion of the DNA helix, separating the two strands. This point of separation is called the ***replication fork***. The two strands are kept separated by ***single stranded binding proteins*** (SSB) which bind onto each of the strands. A group of enzymes called the ***DNA polymerases*** are responsible for creating the new DNA strand, however they cannot start the new strand off, only extend the end of a pre-existing strand. Therefore, before the DNA polymerases can start synthesizing the new strand, the enzyme ***primase*** attaches a short (~60 nucleotides) sequence of RNA called a ***primer***. The DNA polymerases then extend this primer, moving along each strand from the 3' end to the 5' end and adding nucleotides to the 3' hydroxyl group of the previous nucleotide base. The order of nucleotides is retained by matching complementary nucleotides on the template strand.

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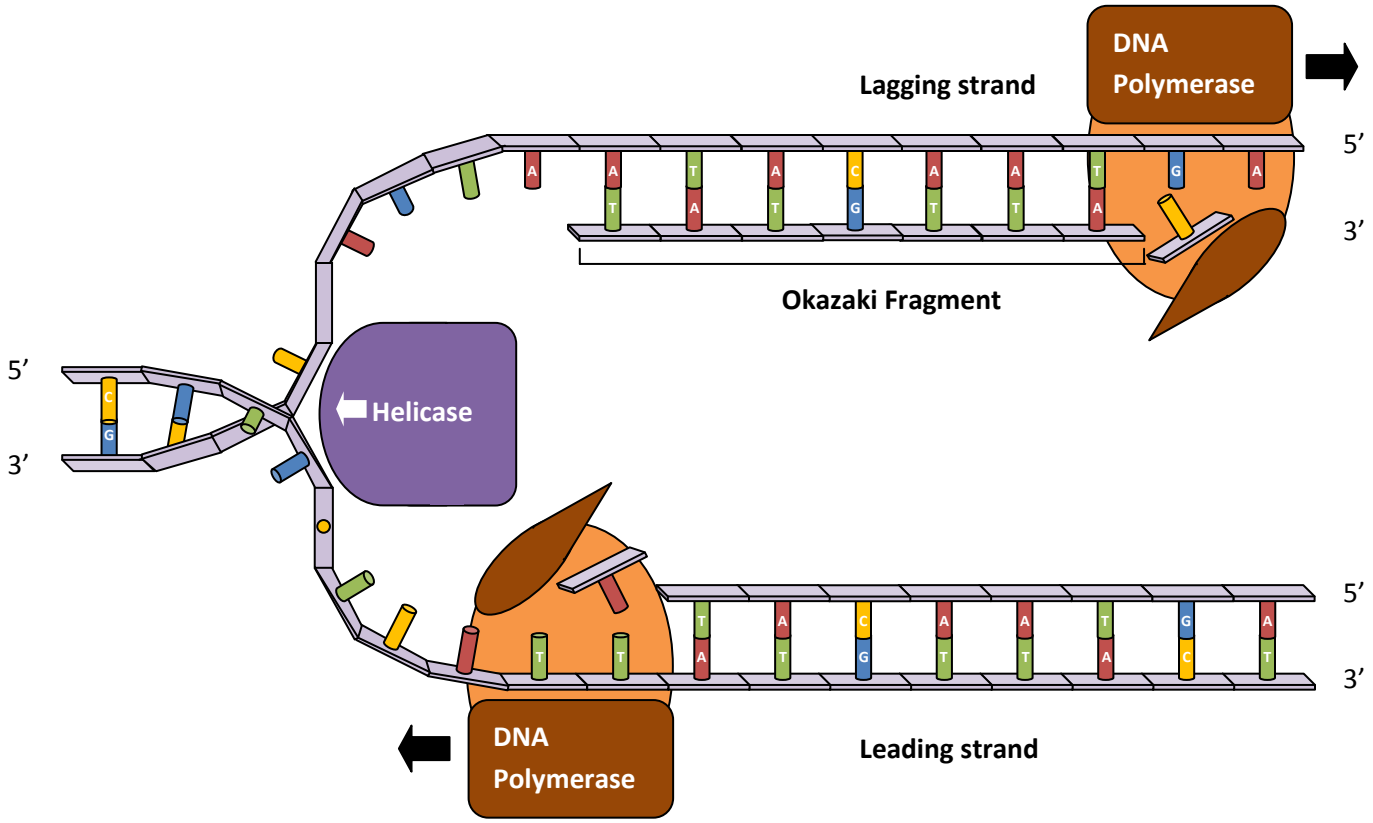

- It’s important to realize that the polymerases can only operate in one direction. This works out for one of the DNA strands (the ***leading strand***) – the polymerase moves along the strand in the same direction as the replication fork. However the other strand (the ***lagging strand***) runs in the opposite direction. As a result the complementary strand to the lagging strand is made in short sections called ***Okazaki fragments***. These sections are then later joined together by the enzyme ***DNA ligase***.

- Once the complementary strand of DNA has been synthesized, the primers are removed by the enzyme ***RNAse H*** and the remaining gaps filled with lengths of DNA by DNA polymerase.

- Some excellent animations of DNA replication can be found here :

  This is a tutorial which takes you through the process step by step.
  http://www.wiley.com/college/pratt/0471393878/student/animations/dna_replication/index.html

  This animation is a computer generated movie showing what the process would look like on a molecular level
  http://www.youtube.com/watch?v=teV62zrm2P0

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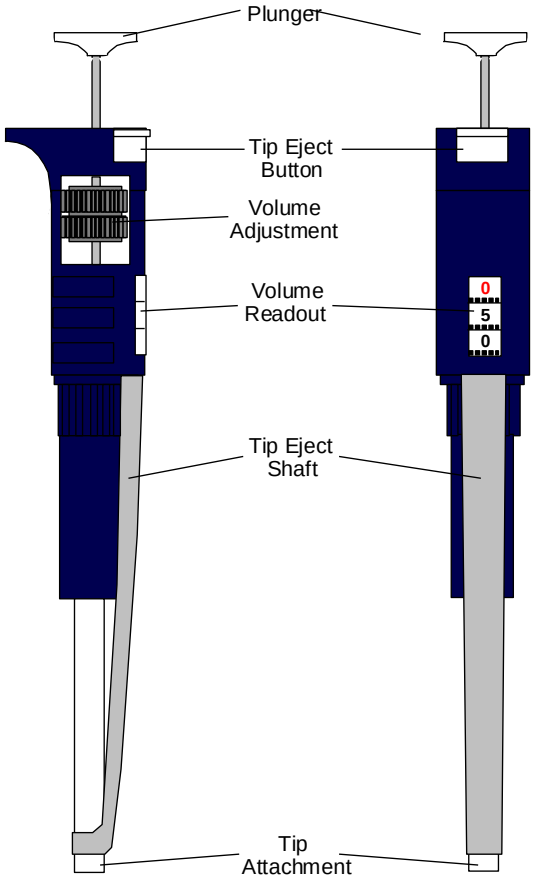
Appendix C : Using a Micropipette

When scientists need to accurately and precisely deliver smaller volumes of a liquid, they use a *pipette* – a calibrated glass tube into which the liquid is drawn and then released. Glass and plastic pipettes have been mainstays of chemistry and biology laboratories for decades, and they can be relied upon to dispense volumes down to 0.1mL.

Molecular biologists frequently use much smaller volumes of liquids in their work, even getting down to 0.1µL (that's one ten thousandth of a millilitre, or one ten millionth of a litre!). For such small volumes, they need to use a *micropipette*.



Micropipettes are called a lot of different names, most of which are based on the companies which manufacture. For example, you might hear them called "Gilsons", as a large number of these devices used in laboratories are made by this company. Regardless of the manufacturer, micropipettes operate on the same principle: a plunger is depressed by the thumb and as it is released, liquid is drawn into a disposable plastic tip. When the plunger is pressed again, the liquid is dispensed.

The tips are an important part of the micropipette and allow the same device to be used for different samples (so long as you change your tip between samples) without washing. They come in a number of different sizes and colours, depending on the micropipette they are used with, and the volume to be dispensed.

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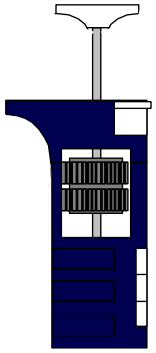
The most commonly used tips are:

- Large Blue – 200-1000µL
- Small Yellow – 2-200µL
- Small White - <2µL

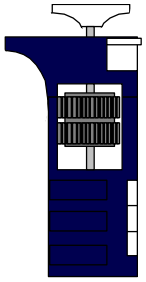
They are loaded into tip boxes which are often sterilised to prevent contamination. For this reason tip boxes should be kept closed if they are not in use. Tips are loaded onto the end of the micropipette by pushing the end of the device into the tip and giving two sharp taps. Once used, tips are ejected into a sharps disposal bin using the tip eject button. Never touch the tip with your fingers, as this poses a contamination risk.

The plunger can rest in any one of three positions:

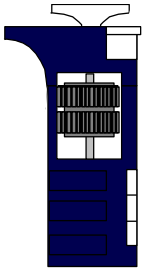
Position 1 is where the pipette is at rest



Position 2 is reached by pushing down on the plunger until resistance is met



Position 3 is reached by pushing down from Position 2



Each of these positions plays an important part in the proper use of the micropipette.

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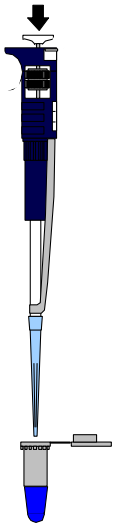
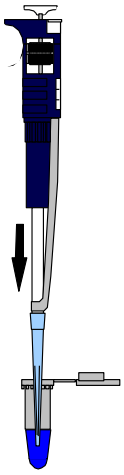
To Draw Up Liquid:

- Hold the micropipette with the thumb resting on the plunger and the fingers curled around the upper body.

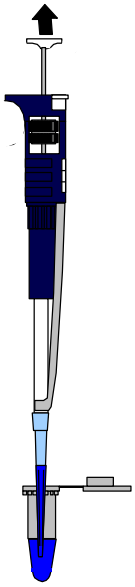
Push down with the thumb until Position 2 is reached.



Keeping the plunger at the second position, place the tip attached to the end of the micropipette beneath the surface of the liquid to be drawn up. Try not to push right to the bottom (especially if you are removing supernatant from a centrifuged pellet), but ensure that the tip is far enough below the surface of the liquid that no air is drawn up.



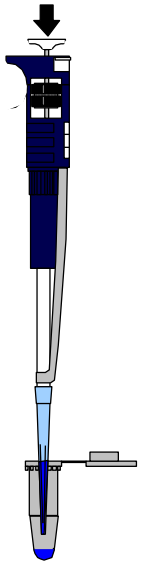
Steadily release pressure on the plunger and allow it to return to Position 1. Do this carefully, particularly with large volumes, as the liquid may shoot up into the tip and the body of the micropipette. If bubbles appear in the tip, return the liquid to the container by pushing down to Position 3 and start again (you may need to change to a dry tip).



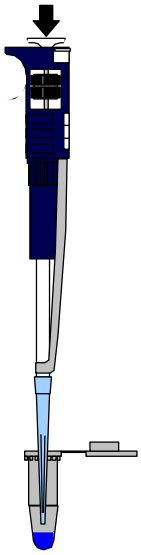
To Dispense Liquid:

- Hold the micropipette so that the end of the tip containing tip is inside the vessel you want to deliver it to. When delivering smaller volumes into another liquid, you may need to put the end of the tip beneath the surface of the liquid (remember to change the tip afterwards if you do this to save contaminating stock). For smaller volumes you may also need to hold the tip against the side of the container.

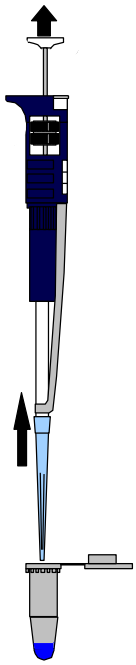
Push the plunger down to Position 2. If you wish to mix two liquids together or resuspend a centrifuged pellet, release to Position 1 and push to Position 2 a few times to draw up and expel the mixed liquids



To remove the last drop of liquid from the tip, push down to Position 3. If delivering into a liquid, remove the tip from the liquid before releasing the plunger



Release the plunger and allow it to return to Position 1



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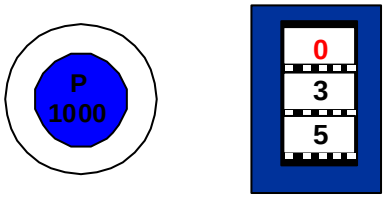
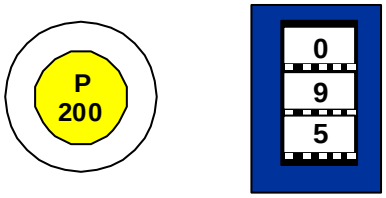
Changing the Volume:

Some micropipettes deliver fixed volumes, however the majority are adjustable. Each brand uses a slightly different method to do this – Gilsons have an adjustable wheel, others have a locking mechanism and turning the plunger adjusts the volume. All have a readout which tells you how much is being delivered and a range of volumes which can be dispensed. Trying to dipense less than the lower value of the range will result in inaccurate measurements. Trying to dispense over the upper range will completely fill the tip and allow liquid to enter the body of the pipette. Do not overwind the volume adjustment, as this affects the calibration of the micropipette.

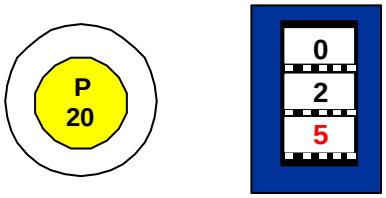
The way to interpret the readout depends on the micropipette used:



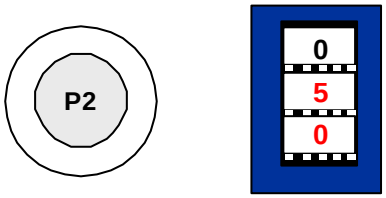
In a 200-1000µL micropipette (e.g. a Gilson P1000) the first red digit is thousands of µL (it should never go past 1), the middle digit is hundreds, while the third is tens. Therefore 1000µL would read as 100, while 350µL would read as 035.



In a 20-200µL micropipette (e.g. a Gilson P200) the first digit is hundreds of µL (it should never go past 2), the second is tens and the third is units. Therefore, 200µL would read as 200, while 95µL would read as 095.



In a 2-20µL micropipette (e.g. a Gilson P20) the first digit is tens of µL (it should never go past 2), the second is units and the third red digit is tenths. Therefore 20µL would read as 200, while 2.5µL would read a 025.



In a 0.2-2µL micropipette (e.g. a Gilson P2) the first digit is units of µL (it should never go past 2), the second red digit is tenths and the third red digit is hundredths. Therefore, 2µL would read as 200, while 0.5µL would read as 050.

Appendix D : Using a Biological Safety Cabinet

Biological safety cabinets are used wherever we want to limit contamination, such as when we are working with pathogenic (disease-causing) organisms or when contamination from outside will seriously compromise our work (e.g. with cell culture).

Unlike Laminar Flow Cabinets (which drawn in air and expel it unfiltered) or Fume Cabinets (which draw in air and expel it outside the room), Biological Safety Cabinets circulate air through a series of high quality filters. Because we want to have a non-contaminating environment inside the cabinet, we need to follow a set procedure whenever we use them.

When Starting Up:

- Remove the metal sash from the front of the cabinet, stacking it on the floor next to it
- Turn the cabinet on – you should hear a rush of air and the light should turn on
- Spray the work area with 70% ethanol and wipe with a paper towel

When Using:

- Gather all of the items you will be using
- All items which enter the cabinet must be decontaminated by spraying with 70% ethanol and wiping with paper towel before being placed inside
- To prevent contamination, briefly spray and rub your gloved hands with 70% ethanol before placing then in the cabinet
- Perform all work with your hands inside the cabinet

When Finished:

- Remove all gear from inside the cabinet
- Unscrew the waste jar from the vacuum line and tip the contents down the sink with a lot of water following it. Rinse and place a squirt of iodine decontaminant into the jar. Replace on the vacuum line
- Spray the work area with iodine decontaminant and wipe with a paper towel
- Spray the work area with water and wipe with a paper towel
- Spray the work area with 70% ethanol and wipe with a paper towel
- Turn the cabinet off and replace the metal sash
- Press the “UV” button – you should see the UV light turn on. It will remain on for 20 minutes (Do not use the cabinet while the UV light is on)

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Appendix E : Glossary of Terms

Anaphase – the phase of mitosis characterised by the separation of the chromosomes into the daughter chromatids and their movement to opposite poles of the dividing cell.

Aneuploidy – unequal or otherwise abnormal distribution of chromosomes following mitosis.

Antibody – a specialised protein molecule produced as part of the immune response which binds specifically to other molecules.

Antibiotic – a chemical agent which kills or inhibits the growth of microorganisms. Antibiotics are sometimes used as selective agents in bacterial culture.

Apoptosis – a type of controlled cell death. Unlike necrosis, the breakdown of the cell occurs in an orderly fashion and cellular components are compartmentalised and removed before they can trigger inflammation or necrosis in neighbouring tissue.

Bacterium – a microorganism with a cell wall but which lacks membrane-bound organelles.

Bases – the four organic molecules which are found in nucleotides. The bases found in DNA are adenine, thymine, guanine and cytosine. In RNA, thymine is replaced by uracil.

Biochemistry – the study of the chemistry of living things.

Biomolecule – a complex organic compound which is made as the result of a biological process. Also called ***macromolecules***, because most are quite large.

Buffer – a compound which helps to keep the pH of a solution stable and constant.

Cancer – a condition characterized by abnormal cell growth and multiplication, as well as migration of affected cells throughout the body.

Cell – the basic unit of all living things. Cells are metabolically active membrane bound bodies capable of reproduction.

Cell Biology – the study of processes which cells use to survive.

Cell Membrane – the outer boundary of the cell. It is composed of a double layer of phospholipids molecules arranged so that the water soluble ends face outwards while the water insoluble fatty ends face inwards. Embedded in this bilayer are various proteins involved in recognition of other substances and transfer of materials across the membrane.

Cell Cycle – the progression of stages which a cell passes through in its growth and development. It consists of G1 (Gap 1) phase, where organelles are produced and the cell starts to increase in size, S (Synthesis) phase, where DNA is replicated so that each daughter cell has a complete copy of the genome, G2 (Gap 2) phase, where the cell checks that all is in order for division, and M (Mitosis) phase, where the chromosomes are separated (mitosis) and the cell divides into two daughter cells (cytokinesis). Following M phase, cells return to G1 phase should they need to divide again. Most cells go from G1 phase into G0 phase, where they carry out their normal cellular functions, as most cells do not need to constantly divide. Changes to the cell cycle can

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lead to a situation where the cells are constantly dividing, a state which may progress to cancer. An understanding of the processes which control the cell cycle can lead to ways to treat cancer, either by stopping the cell cycles of cancerous cells, or preventing cells from turning cancerous in the first place.

Centrosome – a subcellular body consisting of two centrioles embedded in a protein matrix. The centrosome is the site from which the mitotic spindle is constructed.

Checkpoint – a point in the cell cycle where a cell must meet certain conditions before it can pass onto the next stage.

Chromatid – a single copy of the chromosome following DNA replication during S phase. During anaphase, the chromatids are separated.

Chromosome – A length of DNA. Human cells have 46 linear chromosomes, while bacteria have a single circular chromosome.

Culture – the practice of growing cells by providing them with the right temperature and nutrient requirements.

Cytokinesis – the separation of the two daughter cells during cell division.

Deacetylase – an enzyme which removes an acetyl group from a protein.

Dilution – reducing the concentration of a solution by adding more solvent.

DNA – deoxyribonucleic acid – the biomolecule which stores the genetic information in most living things. DNA consists of two strands of deoxynucleotides linked by phosphodiester bonds. The bases in the two nucleotide strands bind in complementary pairs (adenine to thymine, cytosine to guanine) through hydrogen bonds. This gives the molecule the appearance of a twisted ladder, with the sugar-phosphate chains forming the runners and the base pairs forming the rungs. The sugar in the nucleotides which make up DNA is deoxyribose.

Enzyme – a protein which acts as a biological catalyst – it speeds along reactions which would normally be too slow to be useful.

Fluorescence – the emission of visible light by a substance after it has been excited by ultraviolet light.

G1 Phase – the first "gap" phase of the cell cycle following cell division. During G1 phase, the cell carries out its normal functions and duplicates its organelles to replace those lost to the other cell during mitosis. If cells do not need to divide, they may enter a long term "resting" phase called G0 phase. If conditions are suitable and the cell passes the G1/S checkpoint, the cell may pass into S phase.

G2 Phase – the second "gap" phase of the cell cycle following S phase. During G2 phase the cell prepares for mitosis. At the G2/M checkpoint, the DNA is checked for errors before the cell commences mitosis.

Gene – a small section of DNA which contains the information used to produce a protein, or which controls and regulates the expression of other genes.

GFP – Green fluorescent protein – a protein derived from a jellyfish which emits green light when irradiated with ultraviolet light. GFP is commonly used as a tag to localise substances in cell and molecular biology.

HeLa Cells – a line of immortal cells grown in culture which were derived from a uterine tumour removed from a woman named Henrietta Lacks in 1951. They are one of the more widely used mammalian cell lines.

Histone – a family of proteins intimately associated with DNA in the nucleus. DNA wraps around histones to form nucleosomes. This process assists in chromosomal packing and gene regulation.

Histone Acetyltransferase – an enzyme which transfers an acetyl group (-CH3COO-) to histones. This has the effect of unraveling the DNA from the histone and making it more freely available for transcription.

Histone Deacetylase – an enzyme which removes acetyl groups (-CH3COO-) from histones. This has the effect of allowing the DNA to wind more tightly around the histones, thus making it unavailable for transcription.

Histone Deacetylase Inhibitor – a group of compounds which inhibit the action of histone deacetylases, resulting in hyperacetylation of the histone tail and increased gene transcription.

Human papillomavirus (HPV) – a virus which infects the cells of the skin and genital mucosa which is implicated in the development of warts and some cancers.

Hyperproliferation – an abnormal increase in the rate of cell reproduction.

Immunofluorescence – a technique in which a cellular component is localised using an antibody attached to a fluorescent label.

Incubation – a waiting period, to allow a reaction time to take place, or organisms time to grow and multiply.

Interphase – the period between cell divisions during the cell cycle.

Medium – a combination of salts and nutrients dissolved in a liquid (broth) or semi-solid material (plate) in which cells are grown.

Metaphase – the phase of mitosis characterised by the alignment of the chromosomes along the equator of the mitotic spindle.

Micropipette – a device used to accurately and precisely deliver small quantities (<1mL) of liquid.

Microtubule – a subcellular tubular structure made of protein which acts to support other structures within the cell.

Mitosis – the process by which eukaryotic cells distribute chromosomes during cell division. Mitosis consists of prophase, prometaphase, metaphase, anaphase and telophase. The separation of the cells themselves is called cytokinesis.

Mitotic Spindle – a bundle of microtubules formed during mitosis which serves as the frame on which the chromosomes are separated.

Molecular Biology – the study of how chemical processes contribute to living systems. Molecular biology concentrates largely on the nature of DNA and proteins.

Mutation – any change to the normal DNA sequence.

Necrosis – uncontrolled cell death. Damaged cells break apart and released cellular components and agents which may trigger necrosis or inflammation in surrounding tissues.

Nucleic Acid – a biomolecule consisting of a chain of nucleotides connected by phosphodiester bonds. DNA and RNA are nucleic acids.

Nucleoside – a combination of one of the nitrogenous bases (adenine, guanine, thymine, cytosine or uracil) and a five carbon (pentose) sugar – deoxyribose in DNA or ribose in RNA.

Nucleotide – a nucleoside joined to a phosphate (PO_4) group. Nucleotides make up nucleic acids.

Nucleus – a membrane bound body inside the cells of eukaryotes which contains the chromosomal DNA.

pH – the degree of acidity (low pH) or alkalinity (high pH) of a solution.

PLK1 – Polo-like kinase 1 – an enzyme found in a diverse range of animals involved in the regulation of normal mitosis. PLK1 attaches phosphate groups to serine or threonine residues in its target proteins, which has the effect of activating these proteins.

Prometaphase – the stage of mitosis characterised by the breakdown of the nuclear membrane and the attachment of the chromosomes to the mitotic spindle microtubules.

Prophase – the stage of mitosis characterised by the condensation of the chromosomes, the movement of the centrosomes to opposite ends of the cell and the commencement of the formation of the mitotic spindle.

Protein – a biomolecule consisting of polypeptide chains folded up into three dimensional forms. Proteins play many roles in organisms, including being the building blocks of cellular structures, control and regulation of chemical reactions (enzymes), recognition and communication between cells (receptors and hormones) and defense (antibodies).

Ribonucleic Acid (RNA) – a nucleic acid which differs from DNA in that it contains the sugar ribose (instead of deoxyribose) and the nucleotide base uracil instead of thymine. RNA is usually single stranded, although it may double over on itself to create double-stranded regions and hairpin structures. The three major forms of RNA are mRNA (messenger) which is transcribed from the DNA and which carries the instructions for protein synthesis to the ribosome, tRNA (transfer) which bear the amino acids used in protein synthesis, and rRNA (ribosomal) which is found in the ribosome. Recently, attention has been directed to other forms of RNA which play a role in gene regulation : shRNA (short hairpin), siRNA (short interfering) and miRNA (micro).

RNA Interference – a method of gene regulation which targets the action of mRNA post-transcription and pre-translation.

S Phase – the stage of the cell cycle where DNA is duplicated in preparation for mitosis. The centrosome is also copied during S Phase.

Stem Cell – a cell capable of proliferation which can give rise to other stem cells or to cells which can differentiate into mature functional cells.

Stock Solution – a concentrated solution used to store reagents. Stock solutions are usually made to be a certain number of times more concentrated that the working solutions and so must be diluted by the factor to create the working solution. eg. 50X stock must be diluted 1 in 50 before it can be used.

Telophase – the phase of mitosis characterised by the unwinding of the chromosomes and the reformation of the nuclear membrane and other organelles.

Therapeutic – treatment aimed at curing or managing a disease or condition once it has been contracted.

Tissue – a collection of cells which work together to perform a function in the body.

Ultraviolet Light – electromagnetic radiation with a wavelength between 400nm and 10nm.

Working Solution – the solution which is used in a chemical solution. Working solutions may be made up fresh or diluted from stock solutions. They are normally given the name "1X" to differentiate them from their stock solutions.