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Making the Most of Your Microscopy Mini Series

Session 1: Skin Lumps: Whoops, That Didn't Need That….

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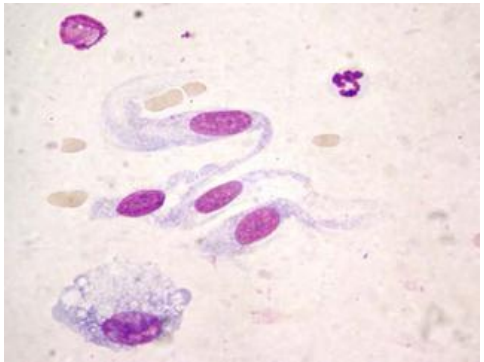


CYTOPATHOLOGY

This is a huge area that cannot be completely covered today, but sampling considerations, fundamental principles and specific common examples will be discussed.

Cytology is very useful as it is minimally invasive and quick, and with good sampling and accurate interpretation, is often diagnostic. Further more invasive, expensive and potentially risky investigations are therefore not always required and therapy can be initiated immediately. Alternatively it can reduce the number of differential diagnoses and therefore help guide further investigations. It is commonly used for superficial or palpable masses (*e.g.* skin) and is good for fluid preparations. Internal structures can be sampled under ultrasound guidance. ***Whenever samples are taken, the size and (palpable or sonographic) nature of the mass should be taken into account.*** A small needle in a large mass will sample only very small areas of the mass, so useful or diagnostic material can be missed. Therefore aspirates from various locations within the mass are recommended. If large enough, ultrasound guidance can be used to target areas. ***Both the centre and periphery of a mass should be sampled.***

Sampling

Impression Smears:

These are done on superficial moist skin structures or areas, such as the mouth, eyelids, vagina or ear canal that can only be sampled using an implement. If accessible, a clean slide is labelled and then simply placed gently over the area. The material naturally sticks to the slide. This 'spotting' can be repeated several times, but care should be taken not to drag the material along as this may damage the cells. This process can then be repeated if required after the area is cleaned with saline to provide representative but slightly deeper samples. By their very nature though, impression smears are very superficial, and so deeper, possibly more significant changes can be missed.

With inaccessible sites, such as the external ear canal, it is best to use a saline moistened swab or cotton bud. This is rubbed/rotated against the area in question and then the swab/bud is **gently rolled** along a clean labelled slide. It should not be dragged as it damages the cells. In this fashion, 2-3 tracks can be placed onto one slide.

Both these styles are left to air dry before staining, most commonly with 'Diff Quik'. They are then examined as for cytological preparations. If speed is essential, after drying, the slide can be dipped once in the blue dye, rinsed and then examined. This produces sufficient staining for certain preparations such as ear smears.

Impression smears can be used for ulcerated lesions as described above, but this is not recommended, as there if often a lot of debris and the sample will only represent the surface of the lesion. Deeper, and usually more significant or diagnostic material, is not therefore seen. These masses are best sampled by fine needle aspiration, the material harvested then being prepared in various ways. The area can be clipped and cleaned, but this is often not required or can be confined to alcohol swabbing only. If the aspirate is for deeper bacterial culture though, the site should be aseptically prepared and allowed to dry thoroughly before aspirating.

Biopsies

Any biopsy tissue obtained for histopathology, if there's enough, can have an impression smear or scrape technique made for cytology. Considerations here are:

- the material should always be gently dried with a (sterile) gauze swab to remove surface blood which then allows the tissue cells to adhere to the slide rather than just blood.
- if the biopsy appears very firm/fibrous or calcified (gritty), scraping the surface gently with a scalpel blade can be better to ensure cells are harvested. This material is then transferred and smeared as described later.
- Alternatively, all the material (if small) can be 'squashed and smeared' – see later.

Fine needle aspiration

The mass is first isolated and 'fixed' using one hand and then one of the techniques described below is used to harvest cellular material.

1. **Needle only** - ***initially it is recommended that the needle is used alone. A 21-23 gauge needle is preferred***. It is held between the thumb and index finger, ***quickly introduced and moved to and fro in the mass without exiting the skin or mass itself. Spinning/rotating the needle on its axis helps to harvest tissue cells.*** The needle is then withdrawn. An air-filled 2.5 ml syringe is attached and the material briskly expelled onto one end of a clean labelled slide. ***Diagnostic material often appears as slightly 'creamy' to blood tinged fluid.*** Grossly the material can appear fatty if a lipoma or adipose tissue is sampled but is not only seen with these masses. This material is then prepared (spread) as outlined later. The sampling process is ideally repeated with a fresh needle. This technique minimises both cell damage and blood contamination. If no material is obtained, it is then best to try a suction method.

2. **Suction –** this is identical to that described above, but when the needle is in the mass, suction is applied by withdrawing the plunger on a (2.5-5ml) syringe attached

before or after needle insertion. It is much easier (and better) to have two people doing this (one holding the mass and one aspirating), as otherwise the needle can move out of the mass when suction is applied. The suction can either be:

intermittent – move the needle, apply suction and release. Redirect the needle and reapply suction, repeating several times.

continuous – suction is applied and maintained whilst the needle is moved to and fro within the mass.

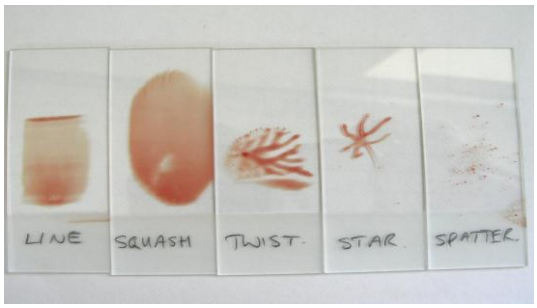
Suction must be released before removing the needle from the mass or the material is lost into the syringe itself. The syringe is removed and filled with air to expel the material as outlined above. Suction methods increase the risk of both cell damage and blood contamination. This is especially true of continuous suction, which can also increase the risk of other tissues around the mass being sampled as the needle is moved. This method though is often essential for firmer masses, especially if they are derived from soft tissue components.

Sample Preparation:

Once the harvest / material has been obtained, it must be smeared gently to create a monolayer, otherwise all or most of the material cannot be examined for cytology.

Once the material has been obtained, there are several ways of dispersing/spreading it to allow staining and examination. ***Crucially the cells must form a monolayer and not become damaged.*** Strengths and weaknesses vary, and very much depend upon the skill of the operator. As with blood films, ***only practise and adaptation allow a consistently good smear to be made.***

The 'Squash' technique outlined below may be the most applicable and useful.



- **blood film technique** – this is identical to that for a blood film and is very good for more liquid, blood contaminated preparations, especially if the tissue cells are known to be fragile. The advancement speed should generally be slower though. For example lymph node aspirates can be prepared like this.

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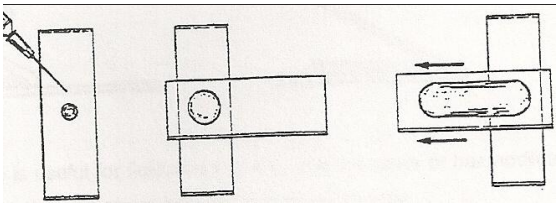
- **line concentration –** this is also the same as the blood film technique above, but when the material has been advanced about 2/3-3/4, the spreader slide is stopped and lifted off. This leaves a line of concentrated cells at one end. It is very useful for fluid and lavage/wash preparations, especially when they have low cellularity. Urine sediment cytology can be prepared this fashion.

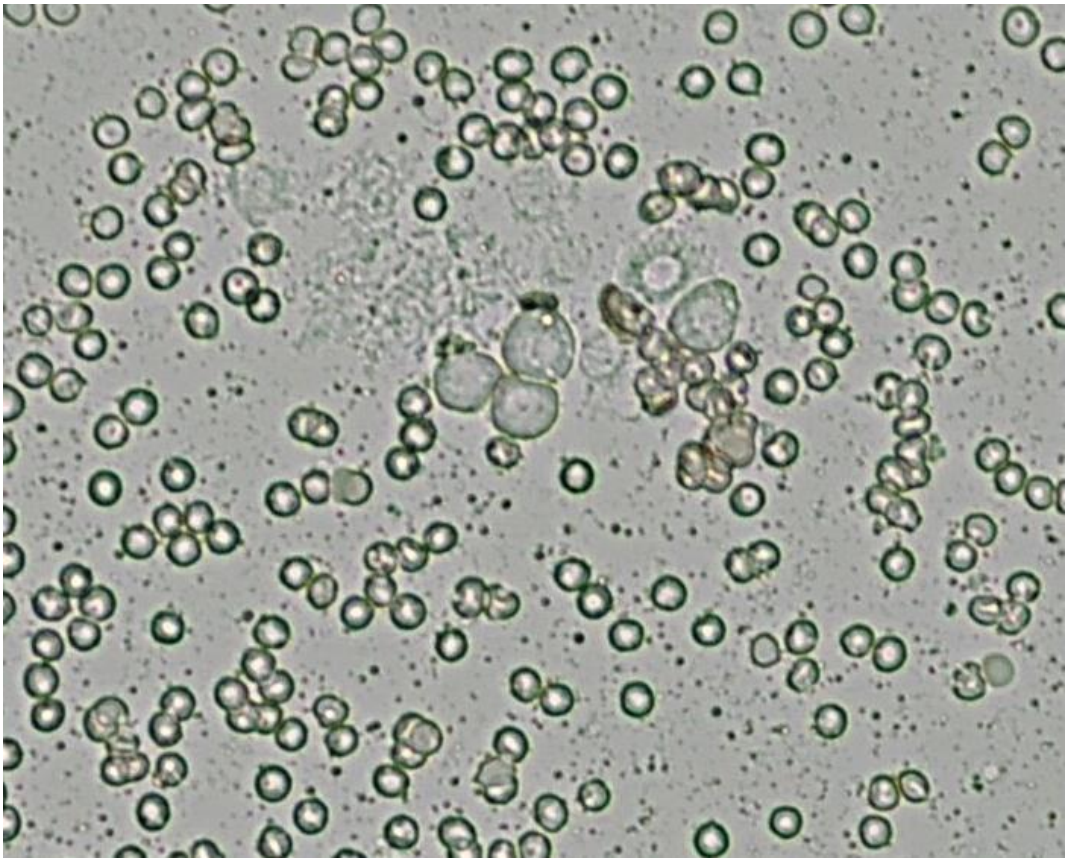
- **'SQUASH' –** If you have steady hands, this can be freestyle. However if not, it can be better to hold (with thumb and index finger at either end) and rest the slide that has the material (A) on a flat bench before starting. Another labelled slide (B) is held at right angles with one end (that nearest your hand) over the droplet of material and lowered onto the material. As slide B touches, the material will naturally spread out. Without exerting any downward pressure, slide B is then smoothly and briskly pulled across over slide A. The majority of material is transferred to slide B, but it is worth using slide A too. Some people perform this with a flourish but this will only damage the cells. Others do it too slowly, allowing the slides to actually 'stick' to each other which also damages the cells. Downward pressure is only used when actual tissue fragments are seen grossly, when gentle pressure is used to squash the fragments and create a thin layer of cells.

- 

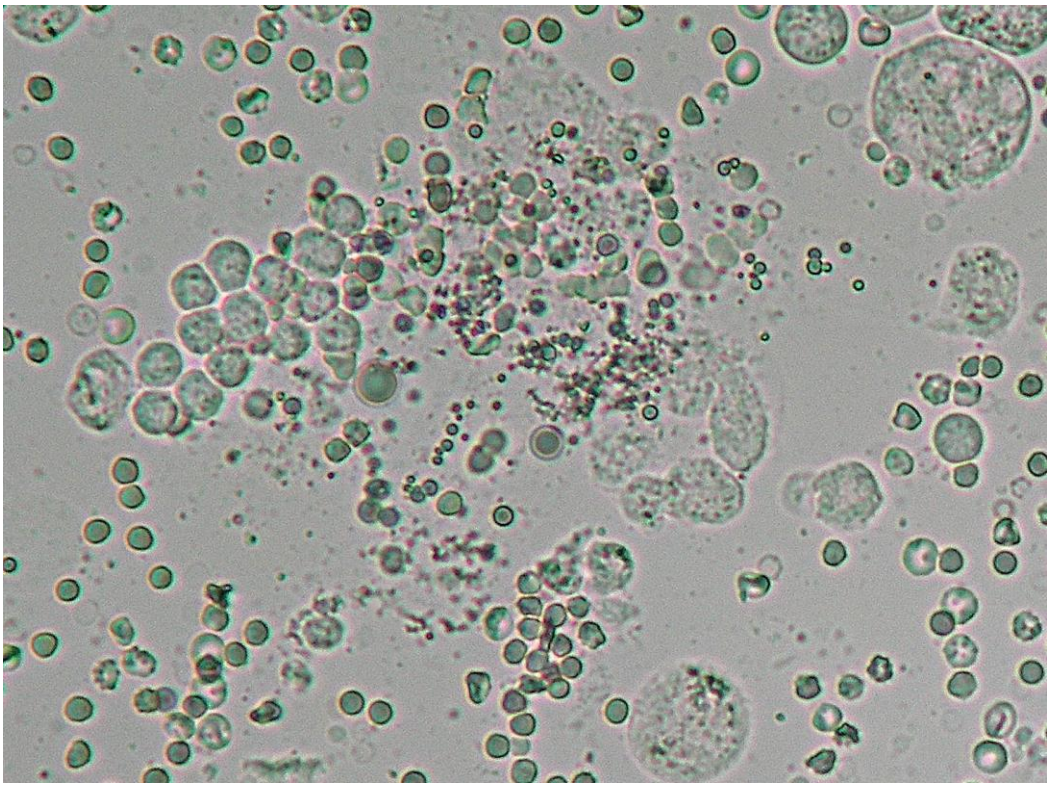
- **Star –** here the aspirate needle is used to drag the material into radiating streaks from the central droplet of material. This is minimally traumatic but unfortunately rarely spreads the cells well, so many areas are too thick for examination. It is not generally recommended, but can be good for (centrifuged/concentrated) fluid preparations that are moderately cellular or grossly turbid in appearance.

SAMPLE CHECKING:

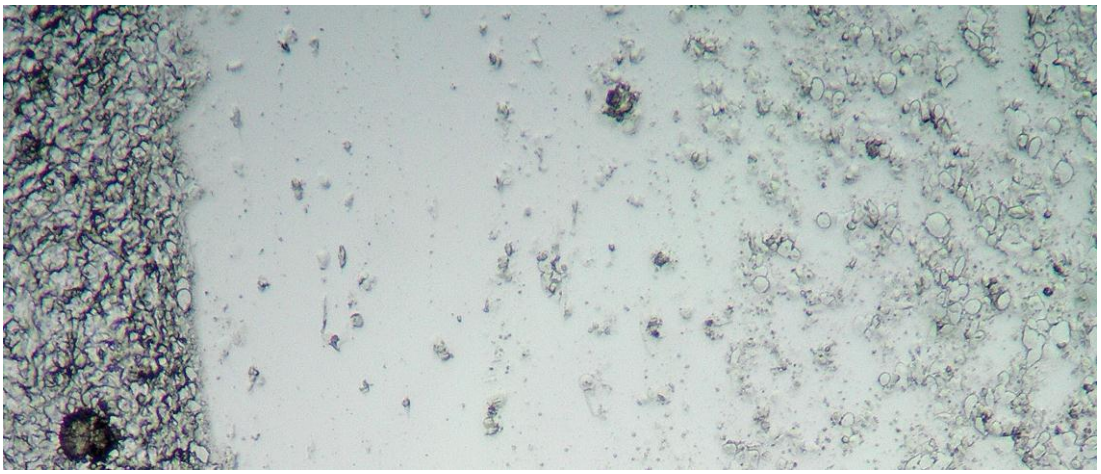
Slides do not need to be stained to check they are well smeared and diagnostically cellular. Practise with blood smears but otherwise cells of interest are nucleated, so at x20, check to see if you can see both the nuclear and cytoplasmic membranes intact, similar to a transparent doughnut. If not, the slide may be too thick, too bloody or too damaged (especially if lots of fine strands or webs are seen). Not staining with a rapid stain prevents cells being badly stained, losing cell detail or not staining significant diagnostic material using a rapid stain. It is much quicker and can be done within seconds of taking the sample while the client waits and requires no 'diagnostic skill', only experience at looking at unstained cells.



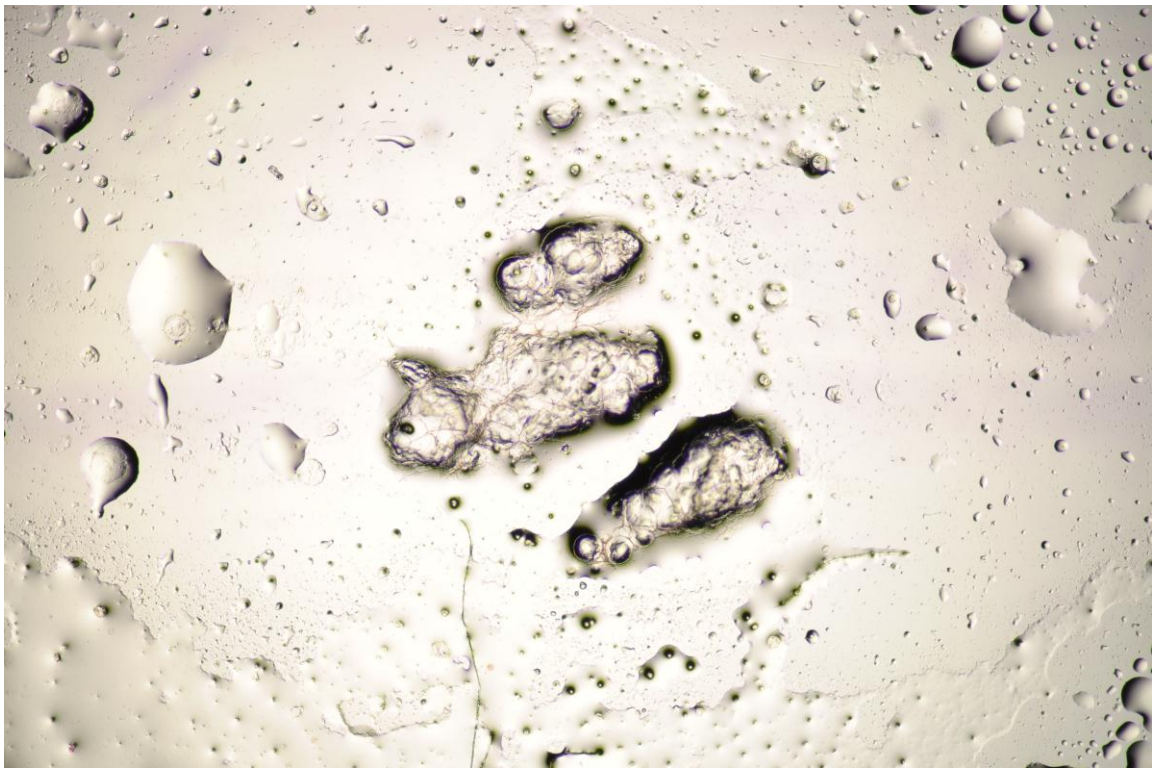
Unstained high power (NCC centre group, others are red cells)



Urine prep unstained – similar, atypical, likely neoplastic TCC



Too thick left side, right side mixed damaged and intact cells



Lipoma unstained: free fat and central adipocyte clusters

Staining

In house staining invariably involves three separate liquids into which the slide is 'dunked' a variable number of times ('Diff Quik'). There are a large number of these stains available. They should be replaced (not just topped up – weekly?) and the containers (*e.g.* Coplin jars) cleaned on a regular basis (monthly or quarterly?), as the staining material becomes depleted with use and stain precipitates form with time ***(golden sheen on the top of the liquid, OR blue granular clumps and brown crystal bars under the microscope)***. Both these factors will prevent or hamper examination of the film and its cells. The first clear liquid is a fixative, the second is an orange dye and the third a blue dye. Typically the manufacturer's guidelines should be followed for the timing and number of 'dunks' required. However, it is worth checking that the colour scheme produced is acceptable for you. For example, eosinophil granules should be orange to pink, neutrophil cytoplasm clear and nuclei dark purple. Often people use too little blue dye or too much rinsing with tap water. ***As a rough rule, after fixing in the first liquid, dunk in the orange dye until an even orange covering is produced on the slide, and then similarly with the blue dye, ensuring no orange dye remains visible.***

Many people rinse any remaining dye off with tap water. This can be fine, especially if gently applied to the back of the slide and not directly to the cells. However, ***it is better to use specific buffered or distilled water as this is gentler to the cells, is consistent and creates little artefactual change.*** Tap water can wash out the dyes and alter the cell shape

artificially. Buffered water is easily made by adding buffer tablets to distilled water. Once rinsed, the slide is allowed to air-dry. As with initial smearing, this can be quickened by judicious use of a hairdryer.

Incompletely or improperly stained slides severely hamper detailed examination of cells. The amount of time in each will vary depending in part on the amount of material – the more material, especially if (too) thick, the longer required in each liquid.

Diff Quik stains are great, especially when time is of the essence such as during intra-operative procedures. However it does have limitations, beyond that of the variable staining mentioned. Relative to other stains such as May Grunwald, Wrights and Giemsa, it shows less fine detail of the cells, especially nuclear detail and chromatin patterns. It can also fail to show granular substances, such as mast cell granules well. This last 'flaw' whilst often quoted rarely prevents identification of a mast cell (tumour) although it does reduce the possibility of suggestive mast cell grading. Conversely, Diff Quik stains can enhance the identification of certain inclusions, such as Distemper viral inclusions in red and white cells.

The majority of in house labs and commercial external labs use water based stains (such as 'Diff Quik' and Wrights) for cytology *etc.* ***It is therefore vital that any slides or fluid taken are not exposed to formalin liquid or even the fumes from biopsy material. If being sent in the post, they should be sent separately***.

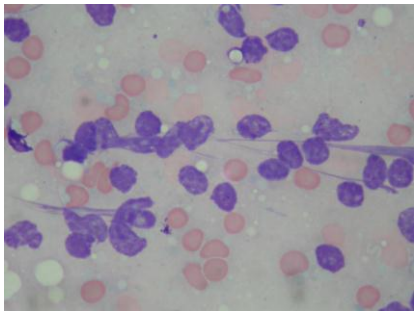
PRINCIPLES OF CYTOLOGY

Cytology uses only a handful of cells to try and diagnose a huge range of diseases and conditions. It therefore requires:

1. good quality samples – is there too much artefact or contamination for a diagnosis?
2. confidence that the sample examined is representative – have we got enough cells to diagnose a disease?
3. a detailed knowledge of normal anatomy and cell appearance - what should we be seeing if this was normal healthy tissue?
4. a detailed knowledge of disease processes – is this sample inflammatory or proliferative, and if proliferative, is it benign or malignant?
5. does the diagnosis 'fit' with the clinical history, signalment *etc*?

1.

One of the commonest pitfalls of cytology is sample artefact. This is often in the form of damage to the cells. Nuclei may remain intact, but without the surrounding cytoplasm, they are difficult if not impossible to interpret. Even the nuclei can be damaged, with only streaks of material remaining. ***Optimal sampling and preservation cannot be overemphasised*.** Contamination can be in the form of adjacent tissues as already mentioned, which can also hamper other diagnostic tests. For example, a tracheal lavage can be contaminated with oropharyngeal material. If inflammation is seen, it is not possible to say whether the inflammation is in the trachea or pharyngeal area. Similarly, this material often contains commensal bacteria, and so culture results can be difficult to interpret. Ultrasound guided aspirates can have too much gel which obscures the cells, or the stain may have too much precipitate which again obscures the underlying cells.



2.

Representation and therefore confidence in a diagnosis is very difficult to assess. It certainly in part reflects experience, but the sampling itself is very influential. The more cells we have, the more likely we are to make the correct diagnosis. Certain clinicians produce very good samples. The history and description of the lesion is very important here. A large mass that has only one smear made from an aspirate is unlikely to be entirely represented in that preparation. Conversely small masses can allow the aspirating needle to exit the mass and aspirate adjacent tissue. This combination of abnormal and normal tissue can make a diagnosis difficult if not impossible.

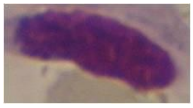
For example: with a history of blunt trauma to an area, proliferating endothelial (mesenchymal) cells is expected and appropriate within a resolving haematoma. Cytologically these can look (very) ‘malignant’ and even dominate the harvest, but are only hyperplastic. Without this historical/clinical knowledge, their behaviour can be interpreted as ‘ectopic’ or inappropriate and therefore neoplastic and possibly malignant. Other features such as organising haemorrhage can provide distinguishing criteria but can be variably present or appreciated.

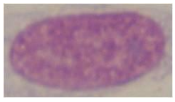
3.

Specific cells and organs are too numerous to describe in a single day. However, all cells and tissues have common features that should always be examined.

Nucleus

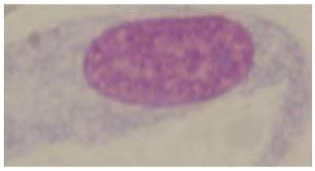
This stains purple with most commonly used stains. It is composed of DNA in the form of heterochromatin (dark inactive DNA) and euchromatin (light active DNA). The varying combination of these two gives the nuclei of cells characteristic textures or appearances (*e.g.* dotted/stippled, smudged or reticular/ropey). Cells in many tissues are 'resting' and so mainly have inactive (heterochromatin) nuclei which appear smooth and dark purple. As the nucleus becomes active and the cell produces (more) substances, whiter areas (euchromatin) are seen. Proliferative tissue cells therefore have more 'open' chromatin, with whiter and darker areas. This activity can be great enough to show as a focal 'hyperactive' point – the nucleolus. This is the area where DNA is actually being read and translated to produce proteins etc. Very active cells have large nucleoli or multiple nucleoli. Nucleolar appearance varies from indistinct clear 'bubbles' to discrete dark patches within the nucleus.
The nucleus is surrounded by a nuclear membrane that is usually invisible and smooth. This membrane is often oval to circular, but can have characteristic shapes.

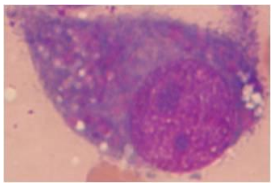




Cytoplasm

This is surrounded by an invisible membrane. However it forms certain shapes that are very useful and whether this shape is discrete and very clear, or poorly demarcated is also crucial. Inside very variable features can be seen. Conversely this time (compared to the nucleus), resting or non proliferative cells have pale smooth cytoplasm. Active cells have darker blue cytoplasm that in some cells can show focal intensity in the form of a pale zone near or adjacent to the nucleus (the Golgi zone). If the cell is productive, product can be seen, as distinct granules (blue, pink, purple etc), indistinct smudges, as a diffuse hue, or in the form of clear vacuoles. If the cell is phagocytic (able to ingest and digest stuff), other cells, infectious agents like bacteria, and debris *etc.* may be seen inside.





Populations

No tissue has only type of cell in it. Therefore the combination and arrangement of cell types can be very important, not only in identifying the tissue/organ, but also whether any of the populations are absent or increased. Certain populations, such as inflammatory cells can be the disease process. Certain tissue types have cellular arrangements that are characteristic.

Background

It should never be underestimated how useful this can be. The appearance of the background is often affected by contamination but can be very useful. Proteinaceous material typically appears variably blue (basophilic) and smooth, whilst glycoproteins/carbohydrates are pinker (eosinophilic). For example, joint fluid is lightly pink and stippled, whereas lymph nodes are lightly basophilic. Chylous effusions have a very light blue background that has numerous irregular vacuoles or clear white spaces.

All of these features will be present in some form in every single cell sample examined. The key is that normal tissue has similar features to proliferative tissue, whether it is benign or malignant. ***There is (probably) no single cell feature that tells you a cell is malignant or not. In other words, malignant features are features that can be seen in 'normal' tissue, but not at that time, to that degree or with such variation***. Normal tissue though is uniform, with cells appearing very similar and regular. Benign proliferation can be almost identical this, but may appear more crowded or 'active'. Malignant tissue can have many similar features, but crucially there will be more variation from cell to cell, in size, shape and features because the proliferation is now uncontrolled and excessive. Unfortunately though, some malignancies (such as leukaemia or lymphoma) are the direct opposite. Here the neoplastic process is very uniform (clonal) and similar from cell to cell (monomorphic).

4.

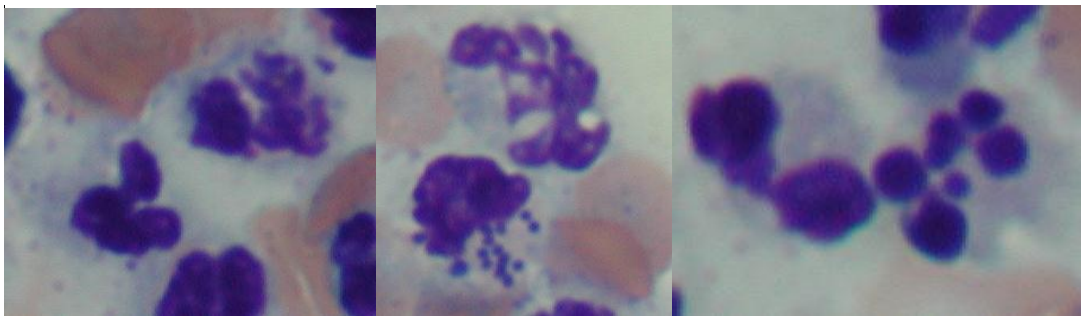
One of the main reasons for cytology is to identify whether a lesion/mass/process is inflammatory, infectious or proliferative (neoplastic). Whenever inflammation or infection is present within tissue, it will cause the surrounding tissue to react and proliferate. As outlined above, physiological reaction to inflammation (hyperplasia and dysplasia) can be very similar to neoplasia (inappropriate or excessive proliferation). When inflammation is present diagnosing neoplasia can be very difficult by cytology alone. Being able to diagnose neoplasia will in part depend on the relative proportions of inflammatory cells to tissue cells. If mostly inflammatory cells are seen and the sample is representative, the lesion is diagnosed as inflammatory. Within this there may be (very) atypical (tissue) cells, but only a few are seen. However, if many atypical cells are seen or they dominate the sample, even if inflammation is present to cause tissue reaction and proliferation, neoplasia can be diagnosed, and if the cells are very variable (pleomorphic), the proliferation is malignant.

Inflammation is commonly broken into three categories based on the dominant or mixture of cells present. This classification can help identify the cause of the inflammation, but can be non specific. Inflammatory cells include: neutrophils, macrophages, eosinophils, lymphocytes, mast cells and plasma cells. Basophils are uncommonly seen/identified.

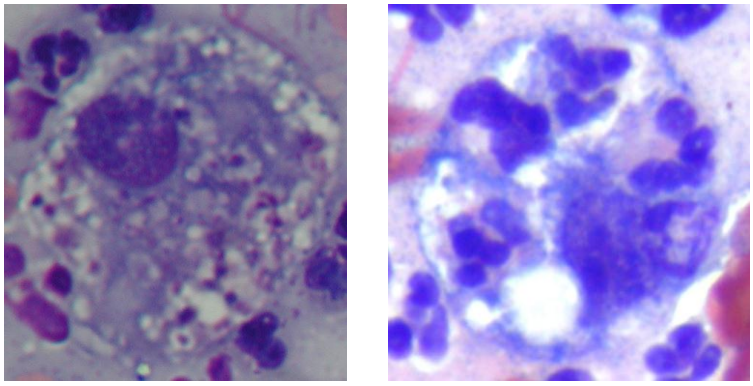
Whenever inflammation is seen, searching for infectious agents is necessary, especially in neutrophils and macrophages, but also within the background potentially. How long do you search? Until you get bored! Alternatively at least 5 mins is a practical guide.

INFLAMMATION

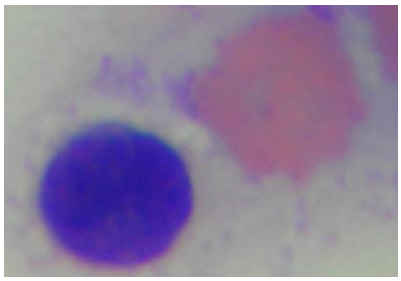
Acute or suppurative – this is dominated by neutrophils, with only low numbers of other mixed inflammatory cells. The neutrophils should be checked for infectious agents, such as bacteria, within their cytoplasm. Bacteria may not be seen, especially if antibiotics have recently been administered. So called 'degenerate' changes though can provide support for an infectious process. These changes involve the nucleus that becomes pale and indistinct, swelling and losing its lobulated appearance. They are not the same as 'toxic' changes seen in the peripheral blood.



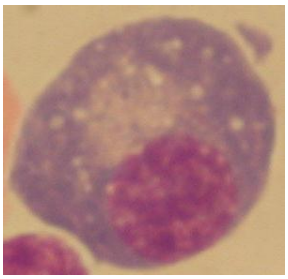
Pyogranulomatous or chronic active – this is when the inflammatory population is very mixed, with macrophages being prominent and about 30-50% of cells seen. Other inflammatory cells can also be present in low numbers. It is non specific, but more commonly seen in foreign body reactions or inflammation/infection that has been present for a while.



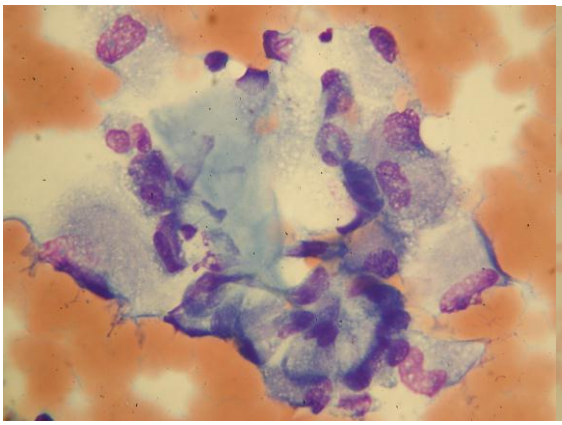
Macrophages

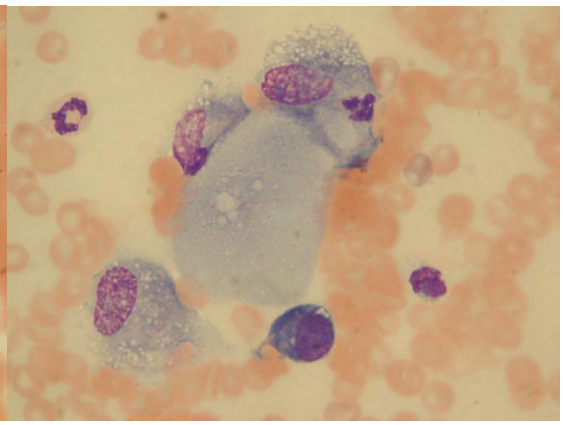


Lymphocyte

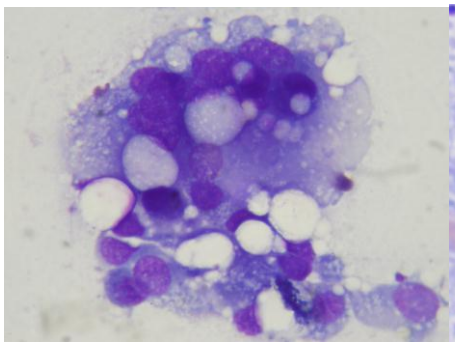


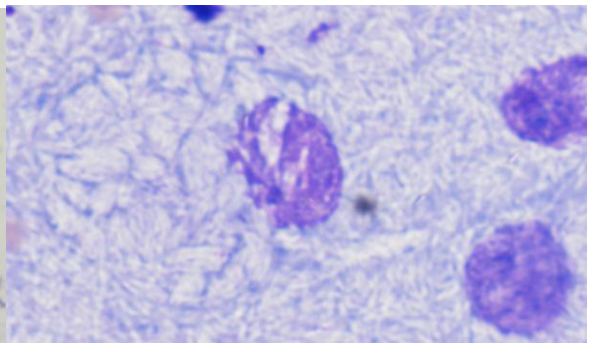
Plasma cell





Granulomatous – this is dominated by mononuclear cells, especially macrophages, along with lymphocytes and plasma cells. Other inflammatory cells are also present, especially neutrophils, but these appear mature and 'non degenerate'. Macrophages are very variable in appearance. Key features include an eccentric nucleus, low nuclear:cytoplasmic ratio, reticular chromatin and abundant very irregular cytoplasm that can contain debris, cells and miscellaneous material. With time and especially foreign body lesions, macrophages can become giant and multinucleate. They can also form loosely cohesive sheets with more blue and smooth cytoplasm, similar in appearance to epithelial cells. These are termed epithelioid macrophages. Granulomatous inflammation can be characteristic of certain infections such as Leishmania and Mycobacteria and also very chronic foreign body reactions (such as around a retained swab).



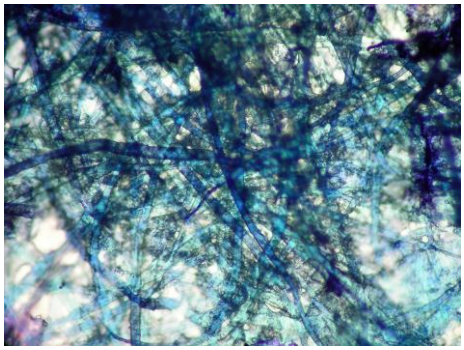


Eosinophilic – this can be dominated by eosinophils, with low numbers of mixed inflammatory cells, often including mast cells. However, if significant numbers (arbitrarily >10%) are present, they are significant and suggest underlying parasitic or allergic diseases most commonly. In cats, focal eosinophilic granulomas can be seen.

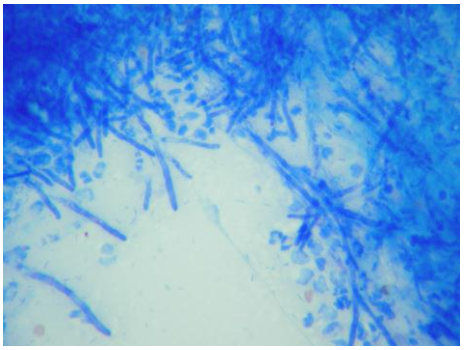
Infectious Agent or Contamination?

Contamination is an unfortunate inherent consequence of cytological preparations that must be minimised (by good sampling, smearing and staining) but appreciated to avoid inaccurate interpretations. As a rule of thumb, to ensure what you are seeing is a genuine 'agent', the following are useful but by no means absolute (see noted exceptions elsewhere in the text):

- ensure a similar appearance can (at least) be duplicated in the smear.
- Phagocytosis – genuine agents creating or forming part of the inflammatory process will be INSIDE the inflammatory cells (same plane of focus as the nucleus).
- Exclude contaminant granular material such as starch, stain dyes and ultrasound gel. Such material is typically more variable, (semi-)refractile, and extracellular or overlying and obscuring.
- Infection is most commonly a single agent and so monomorphic – lots of bacterial shapes and sizes especially if extracellular may be contamination.
- Do the inflammatory response/populations and cell features (such as 'degenerate' changes) correlate with the agent in question? Bacterial infection is typically neutrophilic whilst fungal infection is typically pyogranulomatous to granulomatous. *N.B.* This is variable with the animal's individual response and in part relies on knowing the clinical timescale too.



Paper fibres



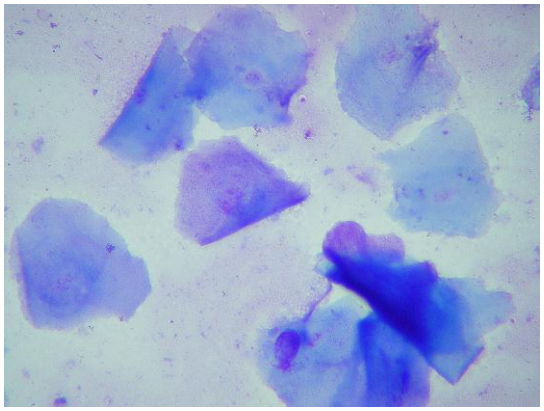
Fungal hyphae

PROLIFERATION (benign and malignant neoplasia)

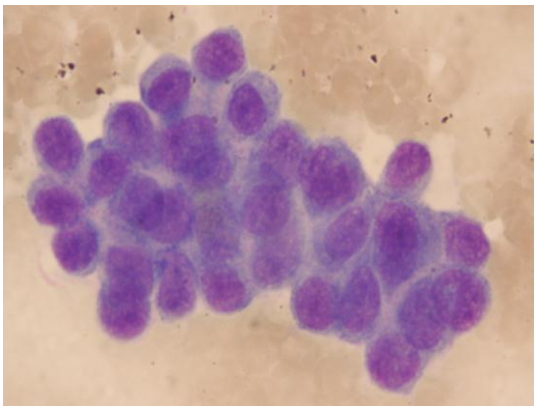
If the sample population is dominated by tissue (*i.e.* non inflammatory) cells, the lesion is likely proliferative. The first step is to try and place the cells into a cell type (not always possible), and then to assess whether they appear benign or malignant. Differentiating benign proliferation from normal (hyperplastic) tissue can be very difficult, and in part relies on the fact that the sample has been taken from an enlarged (and therefore clinically proliferative) area or mass. There are three main cell types cytologically: epithelial, mesenchymal and discrete/round. A rarer less distinct category is neuroendocine, which will not be discussed. Cells may be poorly differentiated, preventing categorisation. **A crucial point is that the guideline features given later are not absolute, with exceptions being seen or overlapping characteristics**. As a rule, the more malignant a mass is, the more likely it is to produce large numbers of cells when aspirated. However, sampling technique also plays a vital role, not only with the number of cells harvested, but also their presentation/preservation, as this can alter cell shapes etc. mimicking other cell types.

Epithelial

These cells typically exfoliate in moderate numbers, originating from the skin or mucosal surfaces. Apart from superficial squames (see below) they are usually in cohesive arrangements – sheets, balls or strands. Cohesion means the cells 'stick together' so that a group of cells can be 'traced with a single line' to include all cells in the group. In the centre of the arrangements, whilst cell boundaries are evident, no distinct white line or space is seen between the cells. They have well demarcated cell borders (sharp clean edges), but shape and features otherwise vary greatly depending on the tissue in question. Nuclei are typically circular to oval. Examples include sebaceous adenoma and squamous carcinoma, the commonest skin lumps though keratinising with minimal cohesive components.



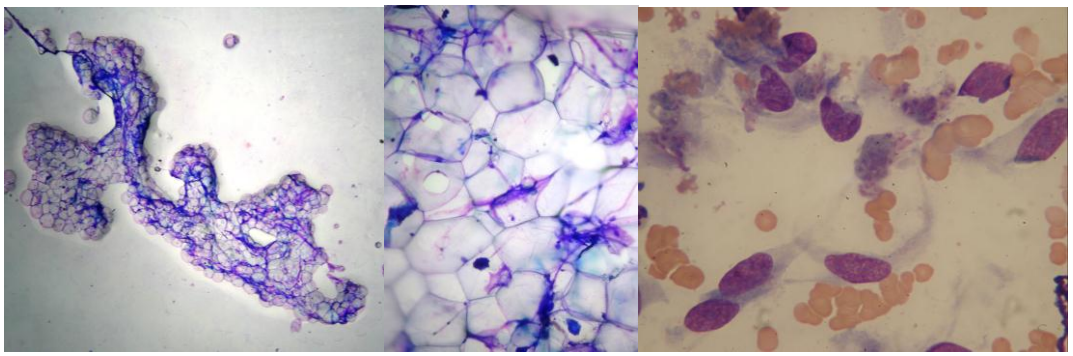
Cystic keratinised epithelial mass



Cohesive epithelial group

Mesenchymal

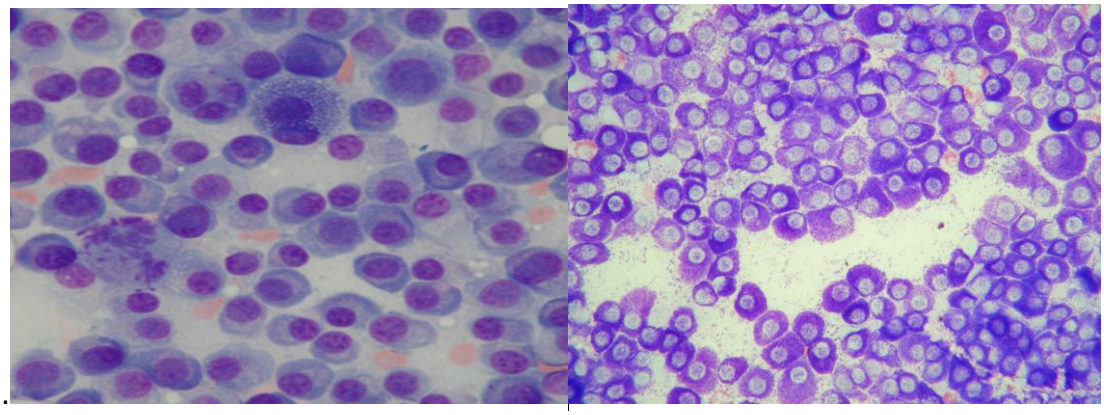
These cells often do not exfoliate in high numbers, and are derived from soft tissue components. Typically their cytoplasm is elongated or fusiform (spindle shaped), with oval to elongated nuclei. They have indistinct poorly demarcated (smudged) cell borders. When present in large numbers and groups, the cells often 'swirl' and fragment, such that a 'single traced line' cannot encompass all the cells in a group. Examples include fibrosarcoma and haemangioma but the commonest is a lipoma.

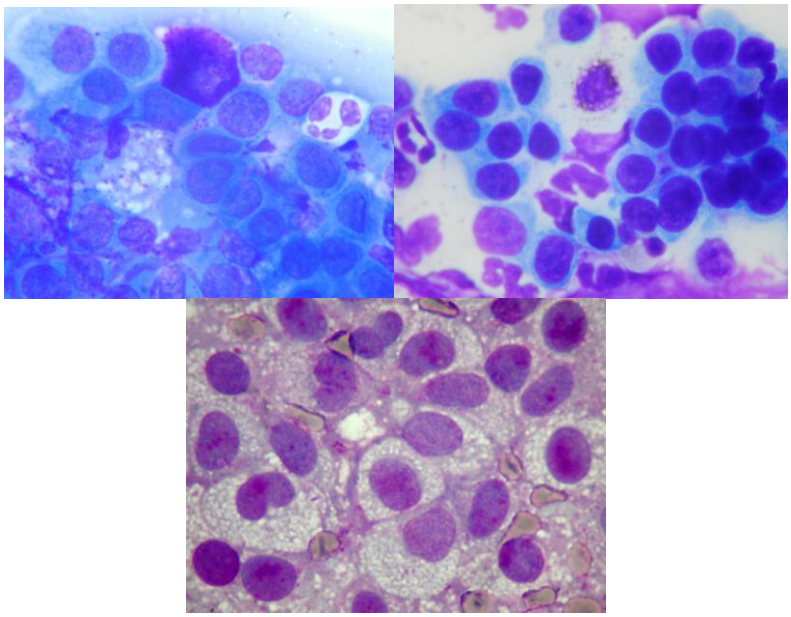


Lipoma (low) (High) Mesenchymal

Discrete or round

These cells often exfoliate in very high numbers. Whilst many cells are round, frequent examples are not, the term 'discrete' more accurately describing their behaviour and appearance. Due to the high numbers, cells are often present in groups that appear 'cohesive'. However, each cell can be individually traced and it is not possible to use a single line to outline a group without 'doubling' up. Cell borders are clean and sharp but not angular (unlike epithelial cells). Features otherwise are very variable and specific to the cell of origin. Commoner examples include mast cell tumour, plasmacytoma and histiocytoma.





Malignant versus benign:

Neoplasia is the formation of new tissue that is inappropriate or uncontrolled. It is traditionally (and probably simplistically) separated into benign versus malignant.

Benign:

Arguably this cannot be diagnosed by cytology in terms of its differentiation from hyperplasia. However, a hyperplastic response should (by definition) have an inciting cause evident cytologically or historically. Benign features are:

N:C ratios are low and consistent

Cells are monomorphic with little variation in features and size

Nucleoli may be visible but are also monomorphic and usually single.

Malignant:

Typically increased pleomorphism, cellularity and 'anarchy' but features can be very subtle. Ectopic proliferation also (*e.g.* cutaneous lymphoma). Features include:

Nucleoli – more prominent, variable size and shape, may be multiple

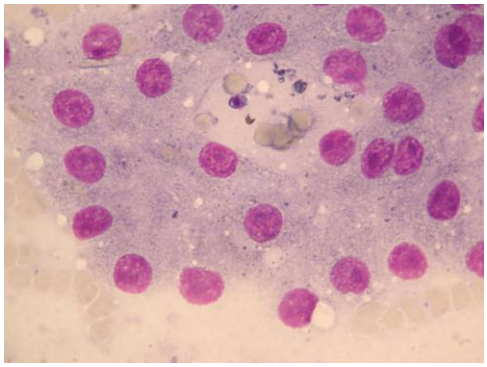
Nuclei – vary in size and shape and number, variable or coarse chromatin pattern

High and/or variable N:C ratio, especially within multinucleate cells.

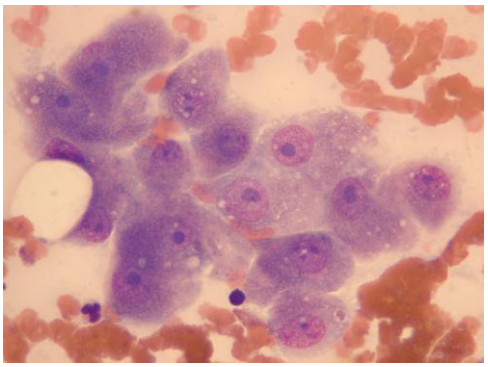
Angular shapes – cells, nuclei and/or nucleoli

Macro or giant forms – cells (cytomegaly), nuclei (karyomegaly) and nucleoli (macronucleoli – greater than a RBC in size)

Mitotic figures – especially if atypical.



Mature hepatocytes

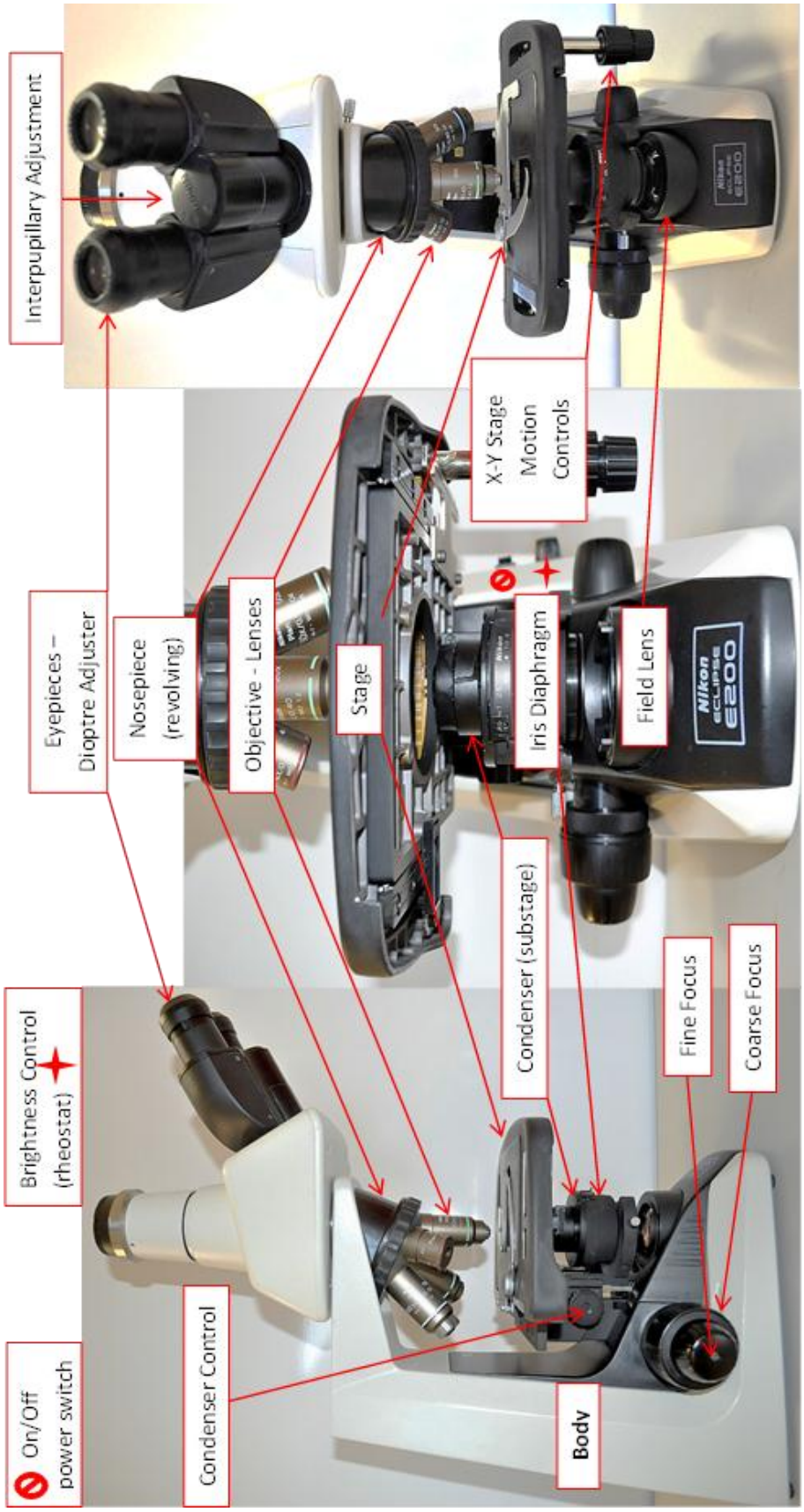


Hepatic carcinoma

5.

Whenever you make a cytological diagnosis, it should be made within the context of the history, signalment *etc.* This not only gives more confidence in the representation as already outlined, but is also an internal quality control check. However, it is also very easy to use this information to over diagnose, providing a diagnosis when actually there are too few cells or other differential diagnoses for the same cell populations. This factor again relies in part on self confidence, but self awareness also plays a role.

Setting up a microscope:



Microscopes without an adjustable field iris diaphragm

1. Turn the lamp on, (adjust the brightness for comfort) and adjust the interpupillary distance so only one image is seen.
2. Place a coverslipped slide onto the microscope stage.
3. Using the x10 objective lens, focus on the slide material. Ensure the eyepiece focus settings are equal and set at 'baseline'.
4. Using the x40 lens, focus on the slide material. Then change back to the x10 lens, and using only your right eye, change the eyepiece focus settings until the image is focussed. Do **not** use the fine/coarse focus control knob. Repeat this procedure using only your left eye.
5. Repeat step 4 using the x40 lens to ensure the eyepiece focuses are perfectly set for your eyes.
6. Set the condenser to just below its highest setting using its focus control knob.
7. Use the desired lens for examination. If the microscope has an adjustable aperture diaphragm on the condenser, this should be set to the same figure as the magnification of the lens (*e.g.* 4, 10, 40 or 100). This aperture should be changed each time the objective lens is changed ideally. If not, a compromise setting for most objective lenses is 40.

The above guidelines allow each microscope to be used to the best of it ability. They do not have to be adhered to, but if not, image quality will be lost and this may hamper examination of material. Once they have been followed once, only steps 1-5 need to be repeated for the microscope to be adjusted to each user, because each person has slightly different strength eyes and interpupillary distances.

The above settings are designed to optimise the microscope for examining cells and cytological preparations. ***However, when examining unstained fresh urine or scrapes etc for parasites, it is advisable to lower the condenser to its lowest setting and close the field iris diaphragm down***. This may require increasing the brightness, but will maximise the refraction and therefore make sediment and parasites very easy to see.

Some References:

BSAVA Manual of Canine and Feline Clinical Pathology 3rd[d] Ed. Editors J. Ristic and E. Villiers.

Diagnostic Cytology and Haematology of the Dog and Cat. 5th Ed. Editors Cowell, Tyler and Meinkoth