

INTRODUCTION

(1) AIM OF INTRODUCTION TO NORMAL HISTOLOGY PRACTICAL CLASSES

The overall aim of the practical classes is to help you understand the biological processes that lead to disease. One of the most satisfying aspects of these classes can be your discovery (by yourself rather than being shown) of the cell and tissue changes associated with a disease. To do this you will examine histological sections either with a microscope or with a computer using scanned image files of histological sections. For those who are not familiar with the microscopic appearances of healthy tissues, the discovery of disease-associated changes can be difficult.

The aim of these two introductory classes is to familiarise you with the histological appearances of healthy tissues so you will more easily be able to find disease-associated changes.

These introductory class sheets and all of the subsequent class sheets can be accessed from the Departmental Web Site ([URL http://www.path.cam.ac.uk/partIB_pract/](http://www.path.cam.ac.uk/partIB_pract/)). The class sheets contain hyperlinks to labeled digital images of the specimens and slides that are shown in the classes.

In all classes, sections of healthy tissues that correspond to the diseased tissues you are examining will always be available at the end of your bench for comparison.

(2) USING YOUR MICROSCOPE

It is very important that you know how to use the microscope properly and feel comfortable using it. The following brief instructions will help, and will be repeated at the beginning of the main component of the course. If, however, you are uncertain about how to use the microscope, or you have difficulty in obtaining a clear image of a slide, please ask either a demonstrator or the classroom staff.

- (a)** If you are tall, put a wooden block under the microscope so that you are more comfortable. (There are wooden blocks on the side of the screen which is near bench F).
- (b)** Before switching on the light, ensure that:-
 - (i) Light intensity is at **minimum**
 - (ii) Low power (x4) objective is in position
 - (iii) Condenser is at upper 'stop' position
 - (iv) Stage is at upper "stop" position [use coarse (larger) focus knob]
- (c)** Switch the light on and **then** increase the light intensity. If the image is **too dark** even with the light control at maximum, check the **aperture** diaphragm of the condenser underneath the slide. It may be closed right down (lever to the right). Move the lever to the left. In general, use the light at the lowest comfortable setting. Please **turn** the light **on and off at low intensity**. Always turn it down to the minimum before switching off.
- (d)** Adjust the distance between the eye-pieces for comfortable binocular vision and note the reading this gives on the **central** scale. Correct each eye-piece scale to the **same** reading and then focus accurately. (You may find you have to re-adjust one of the eye-pieces if your eyes are of unequal focal length).
- (e)** Changing objectives
There are four objectives:
x4 - red (low power objective)
x10 - orange/yellow;
x40 - green/blue (high dry objective)
x100 - blue/black & white (oil-immersion objective). It is rarely necessary to use this objective.

Secure a well focused image with the x40 objective using the **coarse and fine** adjustment knobs. Then turn to the x4 objective and adjust the focus with the **eye-pieces only**. Thereafter, little adjustment should be required on changing from one objective to another.

The oil-immersion (x100) objective: Use only when absolutely necessary with a very small drop of oil. Do not allow the oil to get on any other objective. Having selected a field with the **x40 (high dry objective)**, turn the nose-piece to the **low power** (highest above the slide) so you can place the drop on the slide. Now gently swing the **oil-immersion objective** into the oil. The field will not be in exact focus, so you will have to use the fine focus with great care. **Afterwards**, turn the nose-piece in the **reverse** direction, so the low power objective is again high over the oil. **Do not accidentally bring the x 40 objective into the oil as you do this**. Remove the slide gently and wipe it with a tissue but **use lens paper** to remove the oil from the oil objective. This should prevent the oil from being transferred to the high dry objective.

(3) LOOKING AT TISSUE SECTIONS

- (a) Look with the **naked eye** first against a white background. See if you can identify **distinct areas**? If so note their overall **shapes**, e.g., circular/wedge/cone, **distributions**, patchy, general and **size**.
- (b) Look at the section with the **low power objective**. Scan across the section from one edge of the tissue to the other in order to get an overall impression of the different areas and identify **patterns of cells**.
- (c) Using a **higher power** objective (x10, then x40) home in on representative areas of normal appearance. At this stage, try to **identify cellular and other details**. Always return to low power to finish scanning the section, drawing a sketch at low power and interpreting the **overall picture at low power**.

(4) SCANNED DIGITAL SLIDE IMAGES OF TISSUE SECTIONS: VIEW USING NDP

Instructions for using NDP software:

1. Plug and switch on the PC.
2. Double click on the NDP.view icon on the desktop. NDP.view software is a dedicated viewer for displaying digital slide files (NDPI, VMS files) & lets you open the scanned digital files so you can navigate around the specimen as well as view the specimen at different magnifications.
3. Navigate to a digital slide image file in the appropriate Practical Class sub-folder which is in the Pathology Pt 1B folder & double click on it. NDP.view will open the file.
4. You can navigate around a digital scanned image by using the keys as well as the mouse. The HELP list has all the key functions listed and can be displayed by clicking on the "Toolbar > Icon", "Tool menu > HELP", or by simply pressing the "F1" key.

NDP.view Help:

- Drag the image with the left mouse button to move around.
- Click the left mouse button to centre that point.
- Drag an area to view with the right mouse button
- Zooming in and out of a scan can be done by using mouse's wheel. Push the mouse wheel forward to zoom into the cursor location, pull back to zoom out.
- Hold down the mouse wheel or control key and scroll the mouse wheel to focus (if available)

Keyboard Commands:

- Spacebar: Show overview image.
- S: Show/hide current status
- M: Show/hide maps windows.
- T: Show/hide toolbar
- B: Show/hide scalebar
- F: Enable/disable sharpen filter
- Backspace/Alt+left: To go back to the previous screen.
- Alt+right: To go forward.
- Arrow keys: Move whole screen in that direction.
- C: Copy the current image to the clipboard
- Alt-C: Copy current coordinates to clipboard.
- I: Show/hide image controls
- L: Change language.
- Page Up/Page Down: Focus Up/Down if available
- R: Rotate image (press and release for 90° steps, hold and move mouse for free rotation)
- O: Open a new image
- E: Export the current image to a file
- Q/Escape: Quit
- Number keys change lens:
0: Overview, 1= 1.25x, 2: 2.5x, 3: 5x, 4: 10x, 5: 20x, 6: 40x, 7: 63x, 8: 100x.
- +/-: Change lens up/down

When a new scanned image is opened in the viewer the toolbar is turned on and visible.

5. You can turn the Toolbar on or off by pressing the "T" key or clicking on the **X** in the upper right of the toolbar.
6. The tool menu is displayed by clicking the right mouse button.
7. The magnification can be changed from 1.25x to 100x with the "Toolbar"
8. Multiple scanned files can be opened at once in the viewer by simply selecting "Tool menu > Open". You have the choice to open another scanned image above, below or on either side of the already open scanned image.
9. The scanned images can be locked together (synchronised) so that when one scanned image is moved or zoomed in, the other opened scanned image will also follow the same commands.
10. The current displayed image can be exported as a JPEG, BMP or TIFF or copied by "Toolbar > icon" or "Tool menu > Export or Copy" or "E" or "C" keys respectively.

(5) WHAT IS A TISSUE SECTION?

The tissues sections you will observe in these classes have been made by **fixing** a small fragment of tissue in **formalin** to preserve the tissue structure. This fragment is then **embedded** in paraffin wax, which allows it to be thinly sectioned (sliced) and placed on a microscope slide. The sections you will look at in the introductory classes have been **stained** with a combination of two dyes: **Haematoxylin** stains the DNA and RNA within the cell blue/purple, and **Eosin** stains proteins within and outside the cells pink. Note that it is extremely difficult to obtain identical staining on every slide, so you will become used to observing slides where the strength of eosin and haematoxylin staining varies. Microscope sections present two-dimensional images, harvested in a moment of time, from three-dimensional tissues in which dynamic processes are going on continuously.

(6) THE BASIC STRUCTURE OF CELLS

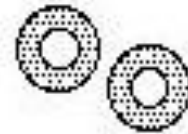
Cells are the fundamental units of living organisms. In the tissues you will be observing they are approximately 10 μM (1/100th of a mm) in size. Cells are bounded by a **plasma membrane**. Within this the pink-staining **cytoplasm** is visible which contains the cellular **organelles** including the blue-staining nucleus. Often details can be seen within the nucleus such as the **nucleolus** and **chromatin** clumps. A good web site to review the basics of cells is <http://www.cellsalive.com/index.htm>. An excellent web site covering normal tissue histology is provided by Dr Teresa Tiffert at the Cambridge University Department of Physiology, Development & Neurosciences http://www.pdn.cam.ac.uk/teaching/resources/1a_histology/index.shtml

OBSERVATION OF SECTIONS FROM HEALTHY TISSUES

NHP1.1 Blood : Normal 86.547 or 96.354

The easiest cells to examine are those that can be observed separately, such as the cells found in blood. This slide of normal blood will introduce you to a wide variety of normal cells.

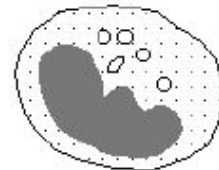
The most common cells on this slide are **red blood cells (erythrocytes)**. These are small cells with a bi-concave cytoplasm. They lack nuclei.



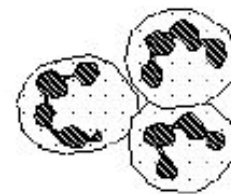
Most of the remaining cells are **white blood cells (leukocytes)**. The size, shape and staining of the nuclei and cytoplasmic organelles varies greatly between leukocyte types.

For example;

Monocytes often have a round or kidney-shaped nucleus and a large pale blue cytoplasm.



Neutrophils have a multi-lobed nucleus (often 3-4 lobes)



Lymphocytes have a large darkly-staining nucleus (with only a thin rim of cytoplasm that is difficult to see in some lymphocytes).



Images of blood cells can be found at the links below on the Departmental teaching server;

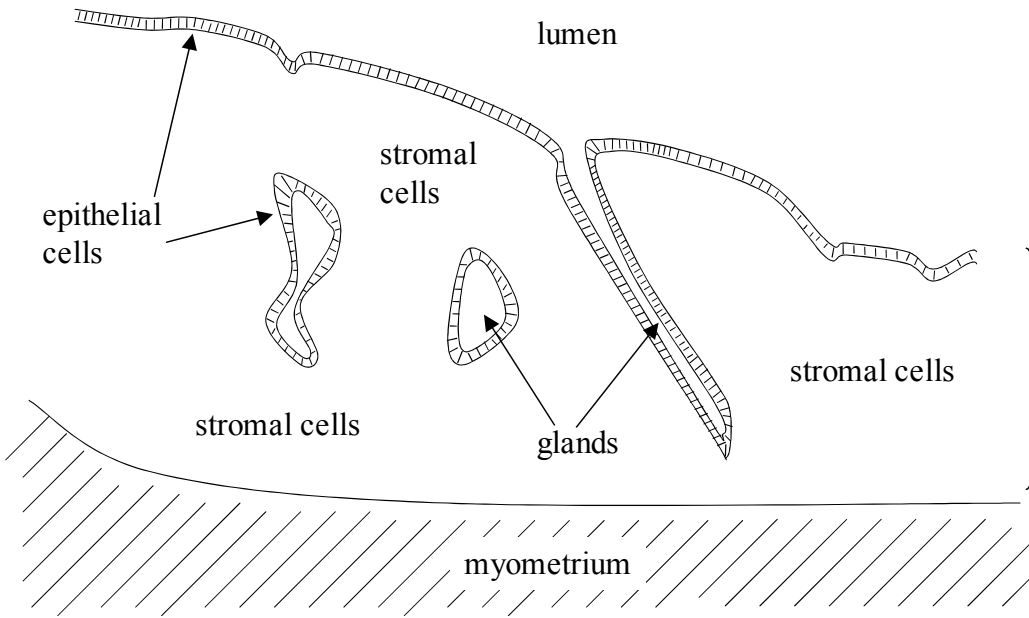
Catalogue Number	Small Image	Image Map	Large Image
N_HL_BF_14.jpg (red blood cells)	Normal Blood	Image Map	Normal Blood
N_HL_BF_09.jpg (neutrophils)	Normal Blood	Image Map	Normal Blood

**NHP1.2 Uterus : Normal
66.217 & 66.218**

The uterus provides an excellent example of a solid organ containing a variety of tissues with different cell types.

The cells within solid tissues can be divided into; (i) the main functional cell type (known as **parenchymal cells** or **parenchyma**) and (ii) supporting cells (known as **stromal cells** or **stroma**). The stromal cells include fibroblasts, blood vessels, nerve bundles, cells of the immune system such as macrophages, and sometimes adipose tissue. Tissues containing predominantly stromal cells are often called **connective tissue**. The stromal and parenchymal cells signal to one another – these signals regulate the survival, division, migration and differentiation of each cell as well as the overall structure of the tissue.

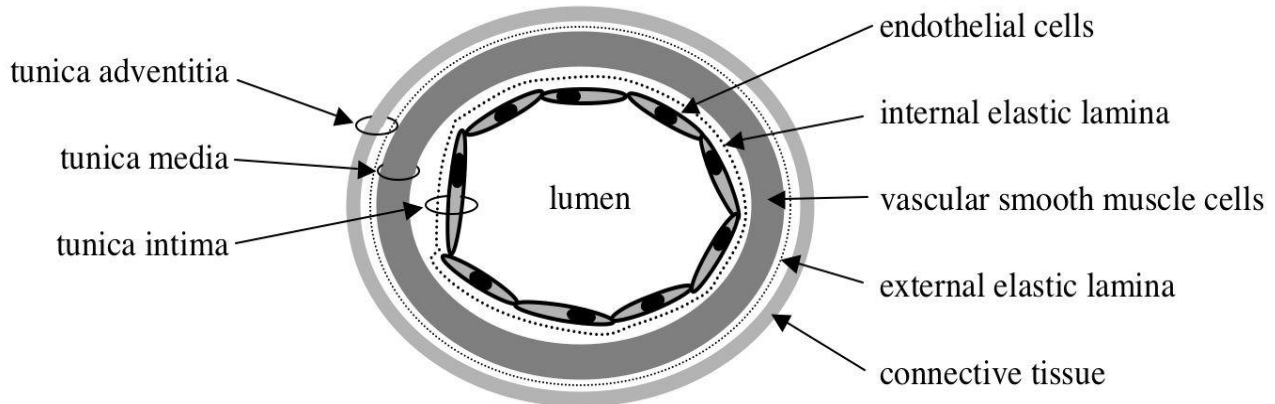
In the uterus the division between parenchymal and stromal cells is easy to observe. The layer of the uterus closest to the lumen of the uterus is known as the **endometrium**. This contains **glands** that open onto the surface. The surface of the endometrium and the glands are lined with **epithelial cells** – these form the parenchyma of this tissue. Underlying and supporting the epithelial cells is a network of many cell types that forms the **stroma** (endometrial stromal cells are spindle shaped with dark blue nuclei). Beneath these stromal cells is the muscular outer layer or wall of the uterus – the **myometrium** (made of spindle shaped smooth muscle cells). With help from your demonstrators try to identify these features on the slide.



Catalogue Number	Small Image	Image Map	Large Image
N_UR_UT_03.jpg	Normal Uterus	Image Map	Normal Uterus

**NHP1.3 Artery : Normal
83.231**

Arteries are made up of 3 concentric cell layers (called tunica). In contact with the lumen are the thin flattened **endothelial cells**. Beneath these is a springy layer of protein called an (internal) **elastic lamina**. Together these inner layers of artery walls are known as the **tunica intima** (or the intima for short). Surrounding the tunica intima are layers of **smooth muscle cells** known as the **tunica media** (forming the muscular wall of the artery). Covering the external surface of the artery is another (external) **elastic lamina** and a layer of connective tissue. Together these outer layers are the **tunica adventitia**. The simplified structural layers of a muscular artery are shown below.



Catalogue Number	Small Image	Image Map	Large Image
N_CR_AY_11.jpg (entire artery wall)	Normal Artery	Image Map	Normal Artery
N_CR_AY_13.jpg (intima and media)	Normal Artery		Normal Artery
N_CR_AY_14.jpg (media and adventitia)	Normal Artery	Image Map	Normal Artery

**NHP1.4 Heart, myocardium : Normal
84.158**

Like arteries, the heart is lined with **endothelial cells**. This endothelial layer is known as the **endocardium**. The cardiac muscle cells above these endothelial cells form the **myocardium**. Cardiac muscle cells are specialized elongated striated muscle cells containing centrally placed nuclei. Their appearance varies depending on whether the muscle cells are cut in longitudinal or transverse section. The myocardium is surrounded by a layer of connective tissue known as the **epicardium** (analogous to the tunica adventitia of blood vessels, often with fat cells). The epicardium is covered with a layer of a lubricated membrane known as the **pericardium**.

Catalogue Number	Small Image	Image Map	Large Image
N_CR_HT_03.jpg (myocardium longitudinal section)	Normal myocardium	Image Map	Normal myocardium
N_CR_HT_02.jpg (myocardium transverse section)	Normal myocardium	Image Map	Normal myocardium

**NHP1.5 Lung : Normal
70.16A**

Most of the lung is made up of thin-walled air sacs known as **alveoli**. These are supplied with air by small **bronchioles**, which are in turn supplied by **bronchi** that are fed from the **trachea**. To allow gas exchange, the alveoli are supplied with blood from the right ventricle of the heart through the **pulmonary vessels**. The lungs also have a second, accessory blood supply from the aorta known as **bronchial arteries**.

Catalogue Number	Small Image	Image Map	Large Image
N_CR_LU_02.jpg (a bronchiole)	Normal Lung	Image Map	Normal Lung
N_CR_LU_08.jpg (alveoli)	Normal Lung	Image Map	Normal Lung

Class NHP1 MUSEUM SPECIMENS

**I Heart : Normal
P80.825**

Catalogue Number	Small Image	Image Map	Large Image
N_CR_HT_05.jpg	Normal Heart	Image Map	Normal Heart

**II Lung : Normal
P82.291**

Catalogue Number	Small Image	Image Map	Large Image
N_CR_LU_18.jpg	Normal Lung		Normal Lung

**III Heart : Normal
P83.250**

Catalogue Number	Small Image	Image Map	Large Image
N_CR_HT_11.jpg	Normal Heart		Normal Heart

TIDYING UP

Before leaving:

Dim and switch off your microscope light. Return the wooden block, if used.

Cover the microscope.

Push your stool under the bench.

Thank you!