African Trypanosomes

Giemsa-stained blood smear of African trypanosomes viewed under the 100X objective lens. The block arrows denote trypomastigote forms of the African trypanosomes found within the blood and tissue of the vertebrate host. The filled block arrows are replicating forms which are in the process of cell division. (Note the two nuclei, two kinetoplasts, and two undulating membranes.) Also indicated are reticulocytes (immature erythrocytes) and platelets. The inset in the lower left shows an enlarged trypomastigote with the kinetoplast (Kt), nucleus (Nu), and undulating membrane (um) indicated.
Trypanosoma cruzi Trypomastigotes and Amastigotes

Blood-stage trypomastigotes of *T. cruzi* (left). Two examples of *T. cruzi* trypomastigotes from Giemsa-stained blood smears in which the kinetoplast (kt), nucleus (nu), undulating membrane (um), and free flagellum (fl) are denoted.

Comparison of *T. gambiense* and *T. cruzi* blood-stage trypomastigotes. Be able to distinguish these two species and know why by the end of the lab sessions.

Amastigotes of *T. cruzi* taken from three different tissue sections (as indicated). The arrowheads in the muscle section denote particularly distinctive amastigotes. The arrowheads in the liver section denote particularly distinctive kinetoplasts. Depending on orientation the kinetoplasts will appear as either dark bars or dots. The nucleus (hn) of the infected host hepatocyte is also obvious in this section. The amastigote labels as 'a' in the spleen section has the nucleus and kinetoplast in the same focal plane. The nucleus and kinetoplast in the 'b' amastigote are in different focal planes and the kinetoplast appears to be on top of the nucleus.
Trypanosoma cruzi Epimastigotes

Epimastigotes of *T. cruzi*. Shown is a Giemsa-stained smear from in vitro cultures of *T. cruzi*. The culture form is the same form found in the gut of the triatomine vector. *T. cruzi* epimastigotes have a prominent bar-shaped kinetoplast just anterior to the nucleus. Extending from the kinetoplast to the anterior end is an undulating membrane. The undulating membrane is sometimes difficult to see in epimastigotes and it often appears that the anterior end of the parasite is twisted. The open block arrows denote epimastigotes in which the features (i.e., nucleus, kinetoplast, undulating membrane) are relatively apparent. The filled block arrows denote dividing forms.
*T. cruzi* in heart muscle. The successive enlargements of the boxed areas are shown at the indicated magnifications. Infected myocytes (i.e., muscle cells) appear as a collection of small dots using the 10X objective as indicated by the arrowheads. The infected host cells are sometimes called pseudocysts. These dots become more evident using the 40X objective. To clearly see the kinetoplasts it is necessary to use the 100X objective lens (i.e., oil immersion). It is often difficult to see the individual amastigotes in the larger pseudocysts which are most evident at the lower magnifications. Distinct amastigotes (indicated by arrowheads) are usually easier to see in the pseudocysts contain less amastigotes. The inset at the lower left is a further enlargement of amastigotes in which the nuclei and kinetoplasts are more evident.
Giemsa-stained impression smear of *Leishmania* amastigotes. (Left) Shown are two infected macrophages. The macrophage nuclei are marked with *. The inset shows and enlargement of some of the amastigotes. The kinetoplasts are denoted with arrowheads and the arrows denote nuclei. The morphology of amastigotes is generally better in impression smears that in tissue sections. (See plates on *T. cruzi* tissue sections.)

(Right) Bone marrow smear of patient with visceral leishmaniasis. Free amastigotes (arrowheads) and an infected macrophage (block arrow) can be seen. Many of the amastigotes are out of focus.
Promastigotes of *Leishmania* derived from in vitro culture. Promastigotes are found within the gut of the sandfly vector. Distinct kinetoplasts and nuclei are evident in many of the these promastigotes. Dividing forms are also evident. Note the variety of morphologies. Different morphological forms representing different developmental stages are also present within the gut of the vector.
Entamoeba histolytica, colonic ulcer

Gross morphology of the human colon and *E. histolytica* ulcers (above). The various features of the anatomical features of the colon and lesions are highlighted. The inset in the upper left shows a schematic representation of the human colon in cross-section with the boxed area denoting the relative position of the section. The mucosal layer, defined in part a layer of intestinal epithelial cells (1), faces the lumen. The lamina propria (2), also called the submucosal layer, lies below the intestinal epithelium. Below this is a muscle layer (3) which consists of a circular muscle layer (inner) and a longitudinal muscle layer (outer). A thin serosa (4), which is not always evident, makes up the outer most layer. Distinct areas within the large lesion are evident. An area with a high level of necrosis (5a) represents the site of the original lesion. This part of the lesion is characterized by extensive tissue damage and very few ameba. Adjacent to this area are areas exhibiting less damage and extensive inflammation (5b). Numerous ameba can be in these areas and represent the progression of the ameba from the original site. Note that the intestinal epithelium has sloughed off above the lesion. Note also that the muscle layer and serosa have been breached (5c). The smaller more recent lesion (5d) demonstrates the classic ‘flask shape’ of the *E. histolytica* ulcer.

Microscopic characterization of the ulcer (next page). Progressively higher magnifications (as denoted with boxes and arrows and objective lens on the right) of different regions of the ulcer are shown. Very few ameba are detected in the highly necrotic area (4X magnification on the left). The ameba are most numerous along the top edge of the large lesion where the intestinal epithelium has sloughed off. Fewer ameba are detected in the area just below the intact epithelium (column on the right) and the smaller lesion, but the damage and inflammation are obvious. Nuclei and ingested erythrocytes will be evident in some of the trophozoites with the 40X and 100X objectives (see below).

(Left) Trophozoites exhibiting nucleus (n) and ingested erythrocyte (e). It is sometimes difficult to distinguish nuclei from ingested erythrocytes because of the section plane or amount of digestion of the erythrocyte. The peripheral chromatin and occasionally the central karyosome will be evident in nuclei from a good section plane. The ingested erythrocytes tend to not have as distinct edges as the nuclei.
**Naegleria fowleri** in Brain

Legend. Trophozoites of *Naegleria* can be found by scanning the foci of inflammation (see legend below) with the 40X objective. The trophozoites are a lavender color and appear to be foamy. Nuclei will be seen in some of the trophozoites depending on the section plane. The halo like appearance around some of the trophozoites is due to a higher level of shrinkage of the trophozoites as compared to the surrounding tissue during the fixation process. Five particularly prominent trophozoites are denoted with the arrowheads. The box indicates the region shown under oil immersion (100X objective). The vacuolated cytoplasm and nuclear (depending on section plane) is more obvious at this magnification. The nucleus is characterized by a large central karyosome and a thin line of peripheral chromatin (enlarged inset). The nuclei will not always be in the section plane.

Legend to figure on other page. The figure depicts a progression going from the view with the naked eye through increasing magnification to the 100X objective (oil immersion). The areas enlarged by subsequent magnification are indicated with boxes and arrows pointing to the view with the subsequent objective lens (i.e., 4X, 10X, 40X, 100X). Two regions of inflammation can be seen when looking at this section with the naked eye (see boxed areas). These foci of inflammation are easily recognized as the darker areas (due to cellular infiltration) using the 4X and 10X objectives. Despite their small size the trophozoites of *Naegleria* can be detected using the 40X (high dry) objective. (See also the figure above.) The arrowheads in the 100X objective view denote trophozoites that are readily recognized. One of these trophozoites is further enlarged to highlight the nuclear morphology and vacuolated cytoplasm. Note the large karyosome (k) and the thin boundary of the nucleus (arrowhead).
Toxoplasma gondii

Toxoplasma in Cat Intestine. (Left) Low power magnification showing the villi and intestinal epithelial cell layer. (Right) Oil immersion view of infected epithelial cells showing various morphological forms. The columnar epithelial cells are quite evident in the middle panel. Note that the parasite lies between the host cell nucleus (n) and the luminal surface of the epithelial cell. Cell #1 is infected with 2 trophozoites. Cell #2 is infected with a meront in which the individual crescent-shaped merozoites are evident. The merozoites can also appear round or oval depending on the angle of the section. Cell #3 contains a macrogamont (i.e., macrogametocyte). Cell #4 is infected with an immature microgamont (i.e., microgametocyte) as evidence by the multiple nuclei. The out-of-focus parasite to the left is probably also an immature microgamont. The nuclei of the mature microgamonts tend to be found around the periphery of the parasite. The far right panel shows three thick-walled refractive immature oocysts which have not yet been released into the intestinal lumen. The arrowheads denote a probable fungus.

Toxoplasma in brain. Shown are successive magnifications (as indicated) of a tissue cyst. Under low power the tissue cysts will appear as round or oval objects with a dot-like appearance (arrowhead in left panel). Small blood vessels (bv) can have a similar size and appearance but are readily distinguished from the tissue cysts at higher magnifications. Individual bradyzoites are apparent at the higher magnifications. A thick wall surrounding the tissue cyst (arrowhead right panel) will sometimes be evident.
Toxoplasma in liver. Shown are successive enlargements of a liver section using the 10X objective (upper left), 40X objective (right side), and 100X objective (insets) as denoted by boxes and arrows. The arrowheads (upper left panel) denote foci of necrosis. These are active areas of infection. Most of the tachyzoites within these necrotic areas are extracellular and are sometimes difficult to detect because of the cellular debris within these lesions. The tachyzoites look like small round or oval bodies and some particularly evident tachyzoites are denoted with arrows in the lower left panel. Host cells infected with tachyzoites can usually be found by looking adjacent to the areas of necrosis as demonstrated by the insets on the upper and lower right corners.