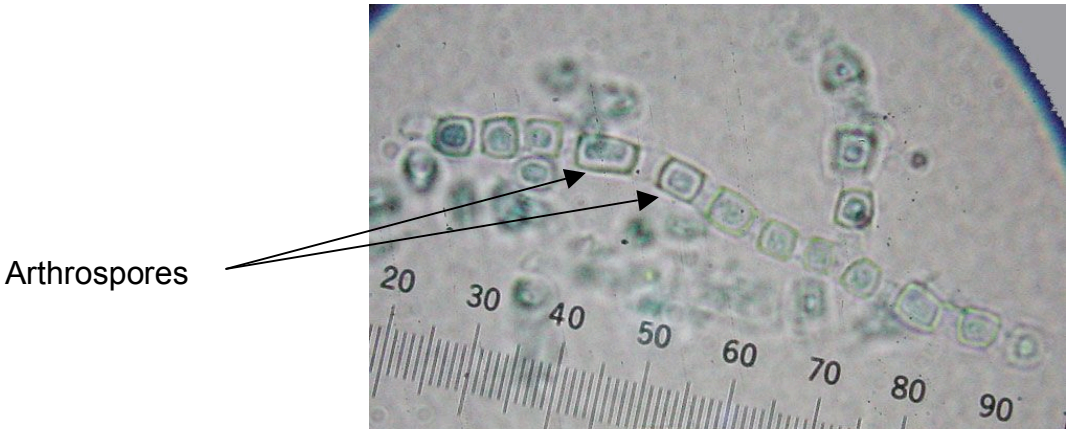
MYCOLOGY TERMINOLOGY

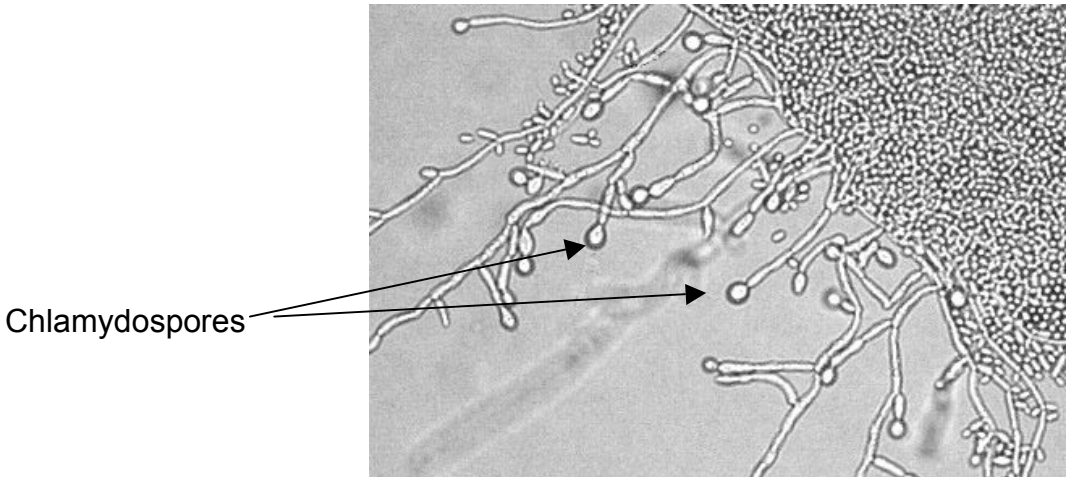
Clinical mycology remains more of a descriptive art than an analytical science. You may find that the identification of fungi requires a greater development of your visual acuity than was necessary in bacteriology. There are also fewer biochemical tests available to aid in the differential identification of fungi. As a result you will spend considerable time in the laboratory visually examining fungal cultures. You will identify characteristic fungal structures by observing colonial growth both macroscopically and microscopically.

A thorough understanding of correct fungal terminology is of critical importance. You may find that the terms used to describe fungi are unusual, at times redundant, and often very confusing. We have attempted to simplify the jumbled jargon by providing you with the following list of terms that will be used most often in the laboratory section of this course. Although most of these terms have already been introduced in the lecture notes, they have been included in the laboratory manual as well.

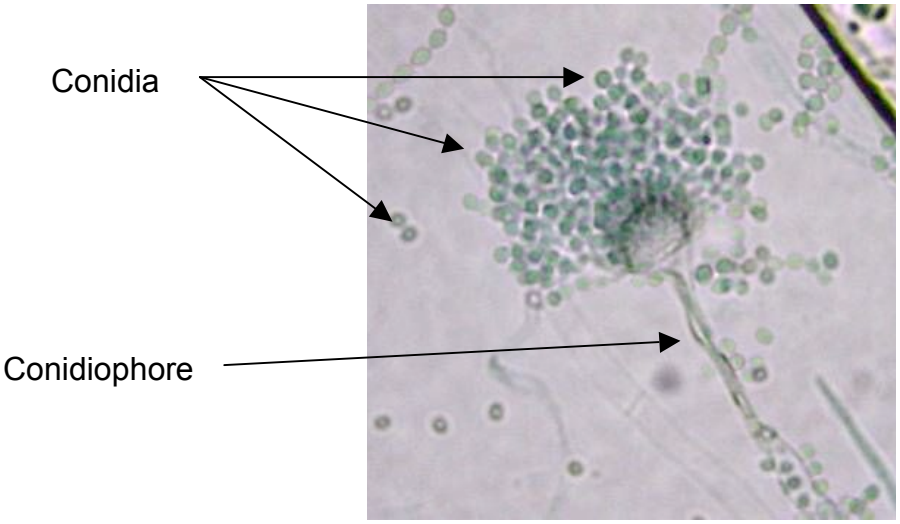
Arthrospores - asexual, thick-walled spores formed by hyphal septation. Arthrospores are released only by fragmentation of the hyphae. The barrel-shaped arthrospores of *Coccidioides immitis* are the infectious stage of coccidioidomycosis.



Chlamydospore - thick, double-walled, resistant, asexual spores that form as enlarged segments either within (intercalary) or at the tip (terminal) of hyphae. Often form under specific nutritional conditions where other spores do not form.



Conidia (singular = conidium) - asexual spores borne externally on hyphae or on a conidiophore; may be unicellular (microconidia) or multicellular (macroconidia).

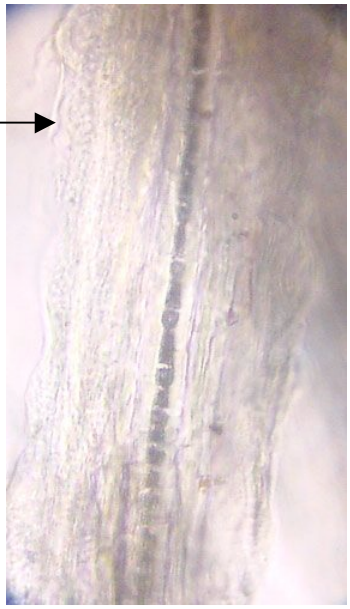


Conidiophore - the specialized hyphal stalk on which conidia develop either singly or in clusters.

Dematiaceous - dark-colored (green, brown, or black) fungi whose hyphae are pigmented. Both the top and the underside of the mycelia will be dark.

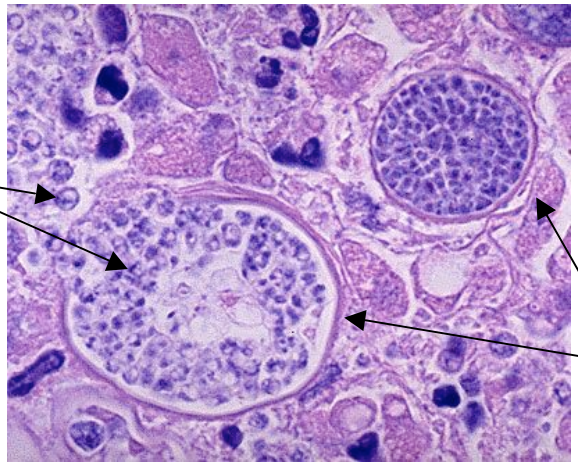
Dimorphic - Having both a yeast and a mold (mycelial) form. Many of the systemic fungal pathogens are dimorphic.

Ectothrix - Dermatophytes that produce a sheath of arthrospores on the external surface of the hair shaft.



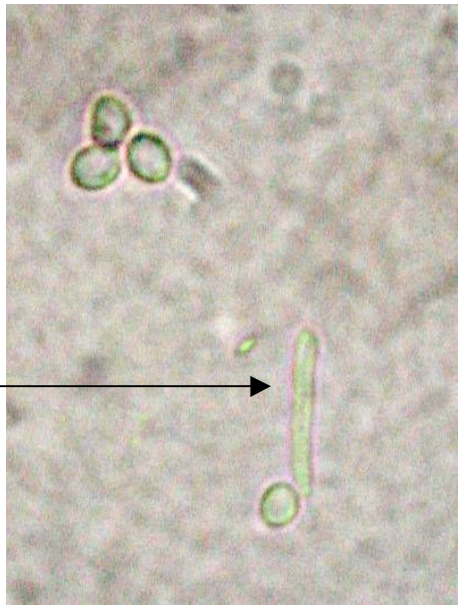
Endothrix - Dermatophytes that produce arthrospores within the hair shaft.

Endospore - Asexual spores formed within a cell (as in the spherule of *Coccidioides immitis*).



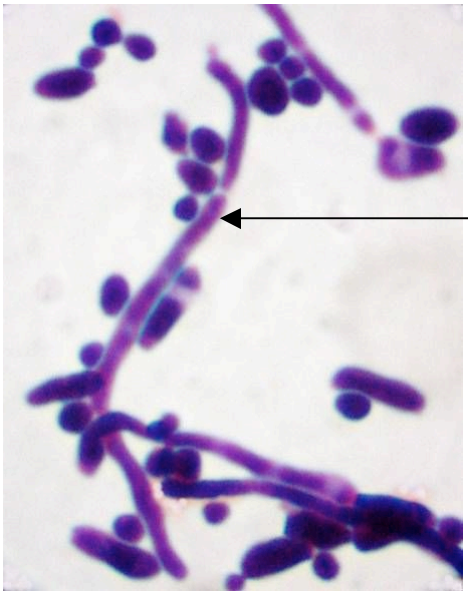
Geophilic - Refers to fungi whose natural habitat is the soil. Use of this term is generally restricted to certain Dermatophytes (e.g. *Microsporum gypseum*).

Germ-tube - The initial hyphal outgrowth of a germinating spore or yeast; especially important for identification of *Candida albicans*.



Germ tube

Mycelium - the intertwined mass of hyphae that forms the mold colony. The vegetative mycelium is composed of those hyphae that adhere to the substrate and absorbs nutrients. The aerial mycelium is composed of those hyphae that grow up from the surface and support the spores.



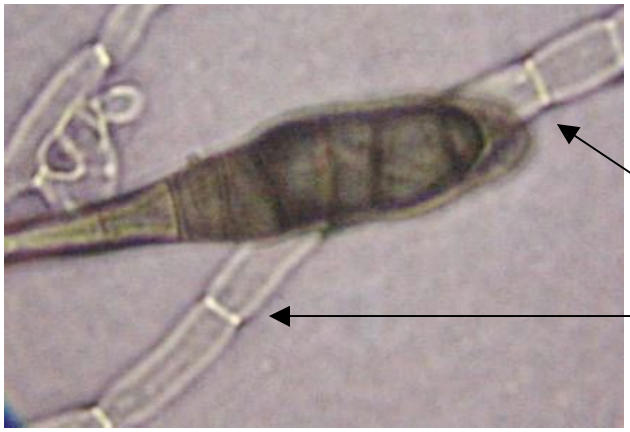
Pseudohyphae - chains of successively budding yeast cells that have complete cell walls, but have not detached from one another.

Pseudohyphae

Rhizoid - rootlike branched hyphae which anchor the mycelium to the substrate; characteristic of certain Zygomycetes (*Rhizopus* and *Absidium*).

Rhizoid

Septate - Cross-walls (septae) that divide hyphae into segments. If there are few or no cross-walls the hyphae are considered to be aseptate.



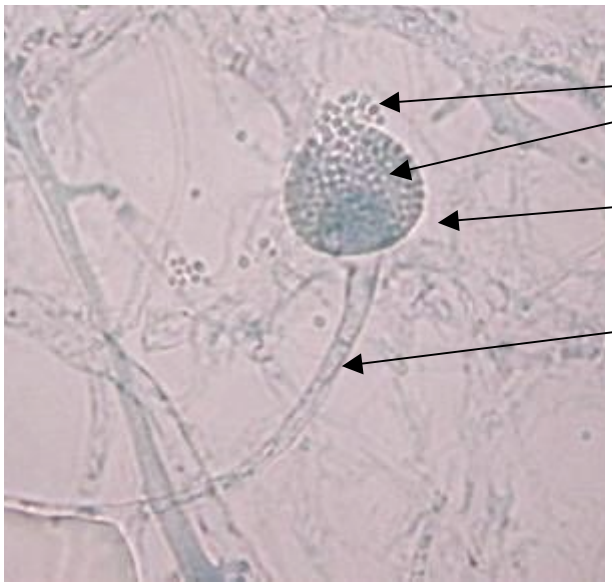
Septae

Sporangia (s orangium)- spherical sack within which asexual

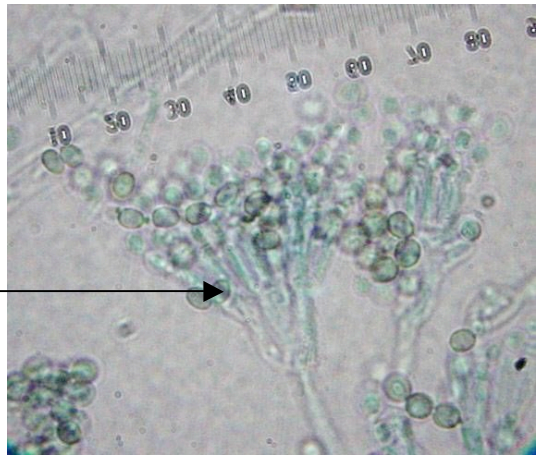
spores (sporangiospores) form by progressive cytoplasmic cleavage.

Sporangiophore - specialized hyphal stalk which bears sporangia.

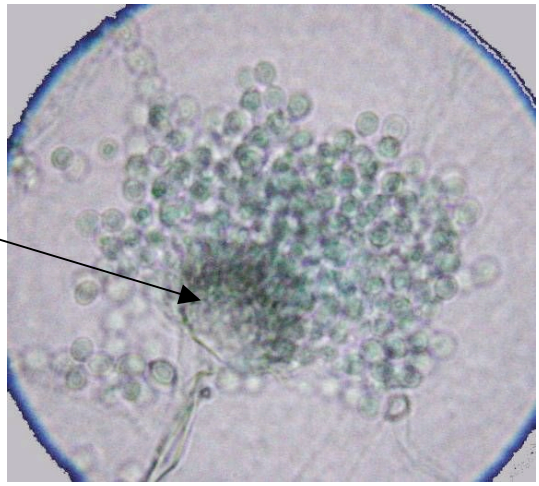
Sporangiospores -- small spores contained within sporangia



Sterigmata -- branched apex of a conidiophore that bears conidia



Vesicle -- swollen apex of a conidiophore that bears conidia

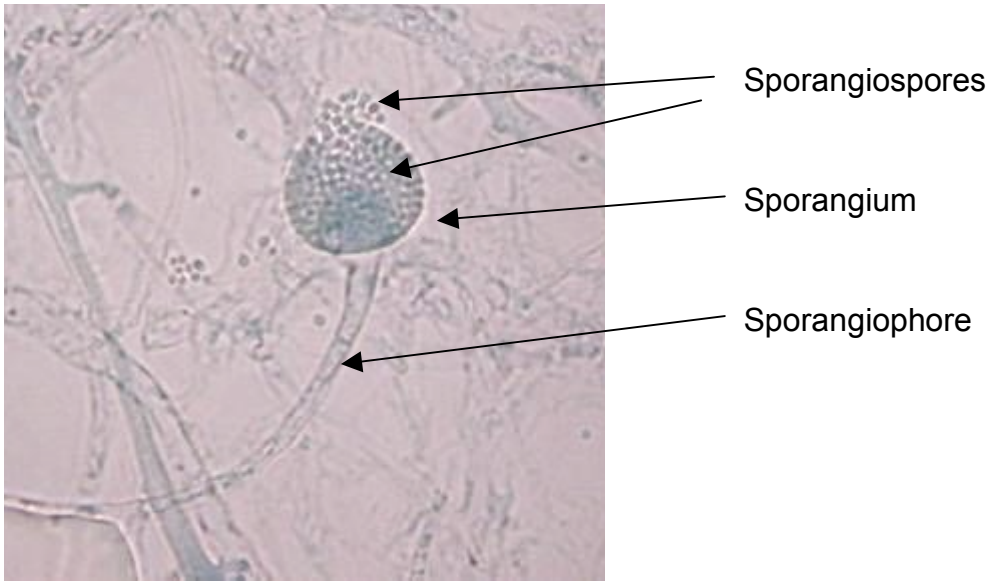


Zoophilic -- refers to certain Dermatophytes that predominantly infect animals as opposed to humans (ex: *Microsporum canis*).

IDENTIFICATION OF FUNGAL ORGANISMS

ZYGOMYCETES

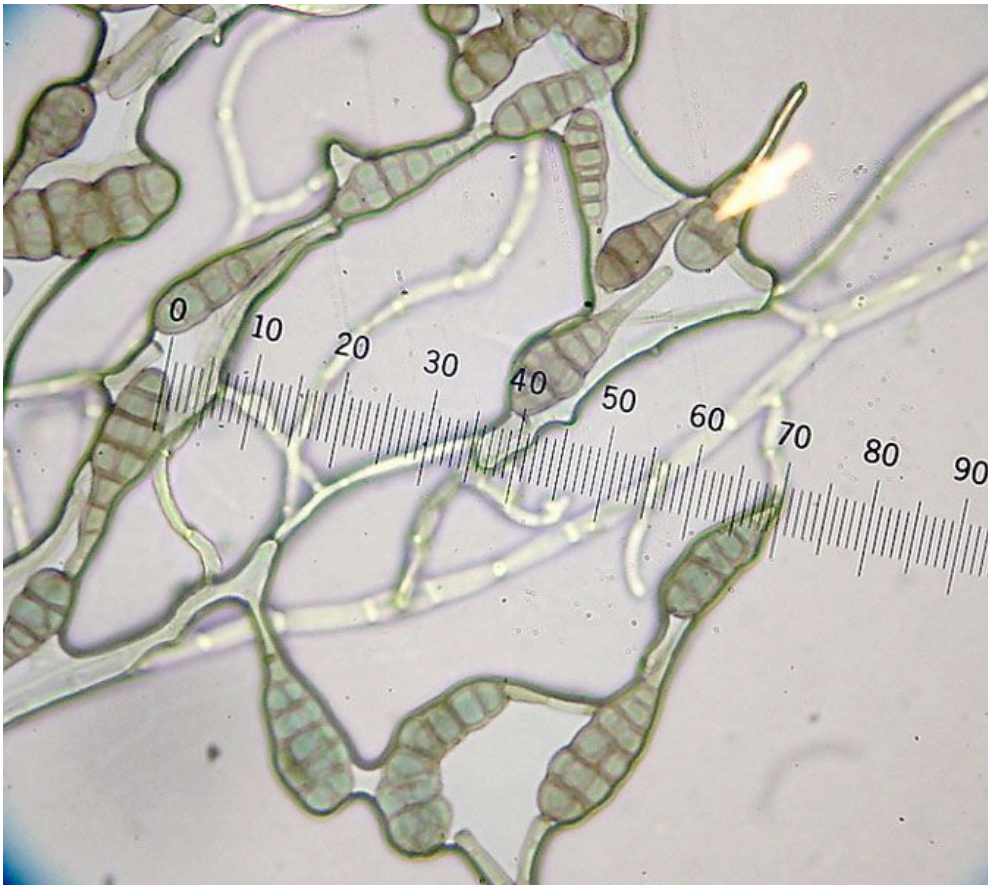
Zygomycetes are rapidly growing molds that typically produce abundant hyphae that look like cotton candy and fill the air space in the tube or plate. They grow very rapidly (perhaps within 1-2 days) both at room temperature and at 37°C (thermotolerant). The Zygomycetes are distinguished from all other fungi by their production of broad, ribbon-like hyphae that usually have few or no septae (cross-walls) that can be seen microscopically. Some species have root-like hyphal extensions that anchor the hyphae. Also protruding from the hyphae are sporangiophores. The sporangiophores bear sac-like structures called sporangia that are filled with small sporangiospores.



Absidia is the representative of the Zygomycetes we will look at in this laboratory microscopically. Other genera of this group that can be opportunistic pathogens include *Rhizopus* and *Mucor*. All of these molds look similar macroscopically. They can be distinguished microscopically by the presence (or absence) and position of rhizoids. The rhizoids of *Absidia*, are positioned between sporangiophores whereas they are found directly below the sporangiophores in *Rhizopus*. *Mucor* doesn't have rhizoids. You **will not** be expected to differentiate these organism based on rhizoid morphology.

DEMATIACEOUS MOLDS

The term dematiaceous refers to molds whose hyphae are pigmented. Because of the pigment, the colonies of these fungi will appear dark green, brown, or black **on both the top and reverse (underside)**. We will provide you with an isolate of Alternaria as an example of this group of fungi. Although it is hardly ever associated with infection, it is a very common contaminant, especially in specimens collected in the barn. Note the "hand-grenade" shape of the pigmented macroconidia. Other opportunistic pathogens of the dematiaceous group include Cladosporium, Phialophora, and Drechslera.

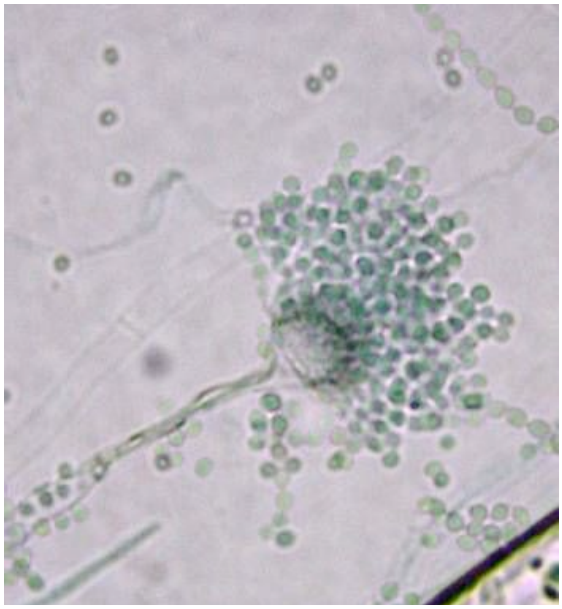


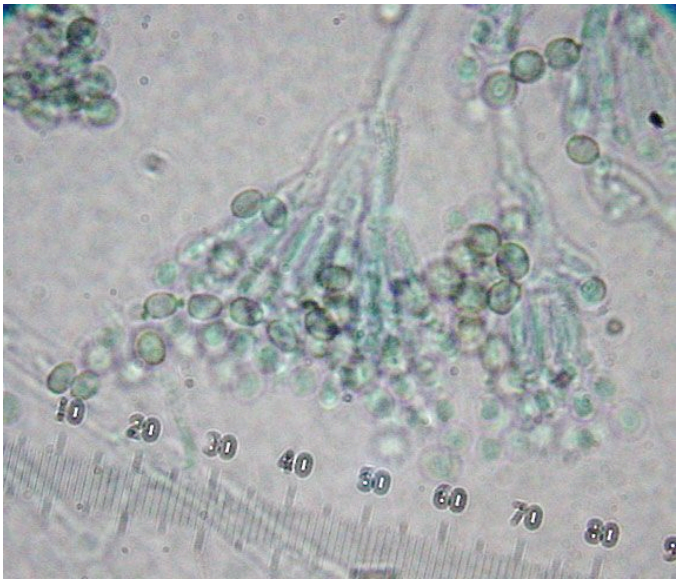
HYALINE MOLDS

Hyaline molds are fast-growing fungi that are **common contaminants and occasional opportunistic pathogens**. Some of these fungi can be relatively common and virulent pathogens (for example *Aspergillus fumigatus* in birds). Examples of opportunistic pathogens in this group include *Pseudallescheria boydii*, *Aspergillus* sp., *Penicillium* sp., and *Fusarium* sp.

Macroscopically, the colonies of these molds are lightly pigmented or non-pigmented. They may be white, light grey, green or light brown. The color is seen chiefly on top of the colony and is due to pigment in the many spores that are produced, not the hyphae. The hyphae are colorless (hyaline) and are septate. These organisms have hyphal projections called conidiophores. Some conidiophores terminate in either a swollen vesicle or a branched sterigmata. The vesicle and sterigmata bear abundant spores called conidia. These structures as a unit are sometimes referred to as "fruiting heads" or "fruiting bodies." Other conidiophores bear conidia singly or in small clusters.

Aspergillus colonies are often white, grey, or green. They may also be yellow, tan, or brown. The colonies of *Aspergillus niger* are black. The septate hyphae of ***Aspergillus* sp**. produce **conidiophores which terminate in swollen vesicles that bear chains of conidia.**

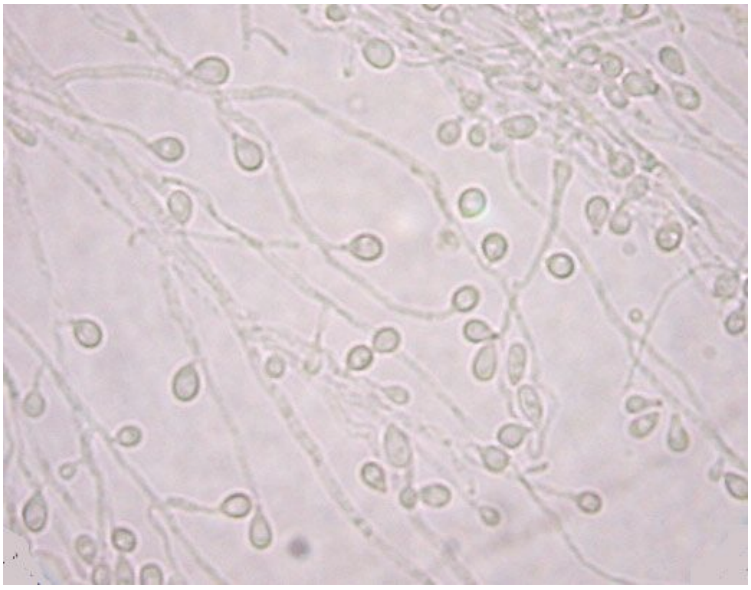




Most colonies of *Penicillium* sp.are some shade of green, but grey, yellow, orange, pink, or white colors may be encountered. The surface of the colonies is powdery because of the huge numbers of conidia. The septate hyphae of ***Penicillium* sp**. produce **conidiophores which terminate in branched sterigmata that bear conidia**.

The colonies of ***Fusarium* sp.** are cottony and have a distinctive lavender color. Their hyphae are septate and produce both macroconidia and microconidia.The microconidia are oval. The **macroconidia are banana-shaped and multi-cellular**. Typically, the macroconidia are produced in small clusters.

The colonies of ***Pseudallescheria boydii*** have a low, cottony surface that appears within 2-6 days. Unlike many hyaline molds, it will grow on mycobiotic agar. The septate hyphae produce single conidia that resemble lollipops. These **lollipop conidia** are **similar to** those produced by an important fungal pathogen, ***Blastomyces dermatitidis***. Fortunately, Blastomyces can be converted to a yeast at 37° (we'll discuss this later) while P. boydii cannot.

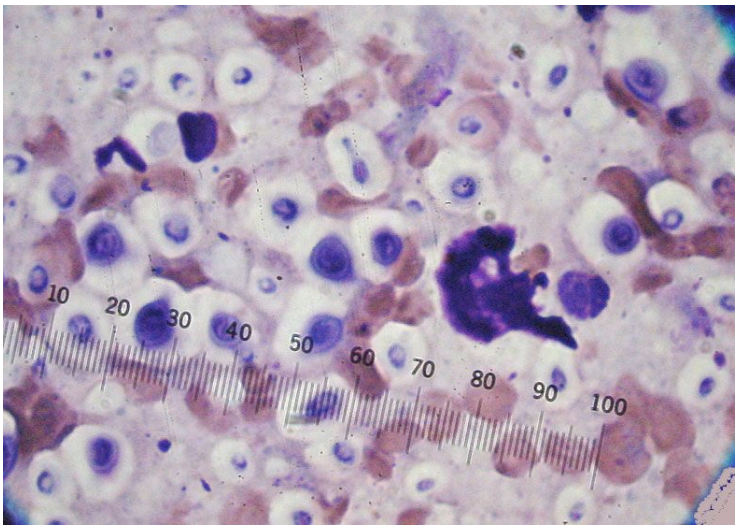


YEASTS

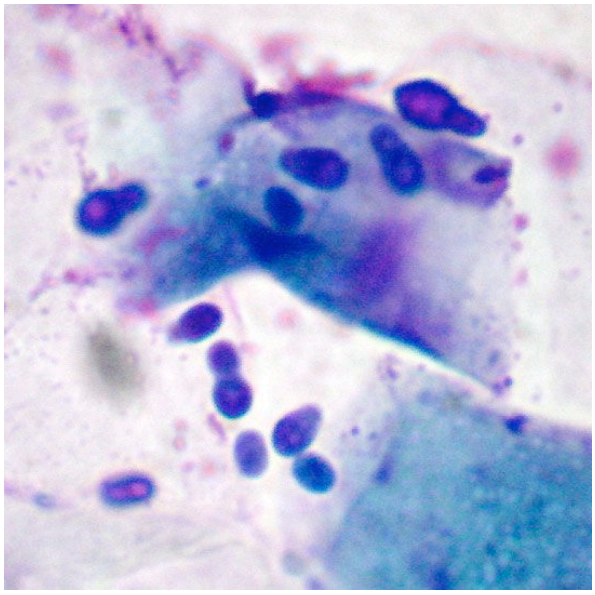
The colonial and microscopic appearance of yeasts differs greatly from that of molds. They grow somewhat more rapidly and may prefer to grow at warmer temperatures (35-37°C) than room temperature. Unlike molds, they do not have a fuzzy colonial appearance because they do not produce hyphae. Yeast colonies are flat and creamy in consistency, similar to many bacterial colonies. They grow a little more slowly, may become a little larger, and may have a more dense convex colonial appearance than bacterial colonies.

Microscopically, yeasts are larger than bacteria and can be visualized easily at 400x magnification (40x objective). Yeasts reproduce by budding -- the cell wall projects outward to form a new daughter cell that eventually pinches off from the mother cell. Some may not separate entirely while budding, forming chains of unseparated yeast cells called pseudohyphae.

Cryptococcus neoformans produces mucoid colonies on Sabouraud agar and brain-heart infusion agar. Microscopically, these organisms are seen as budding yeasts with a narrow neck connecting the mother and daughter yeast cells. The production of a large, mucoid capsule is a unique feature of *Cryptococcus* among fungal organisms.

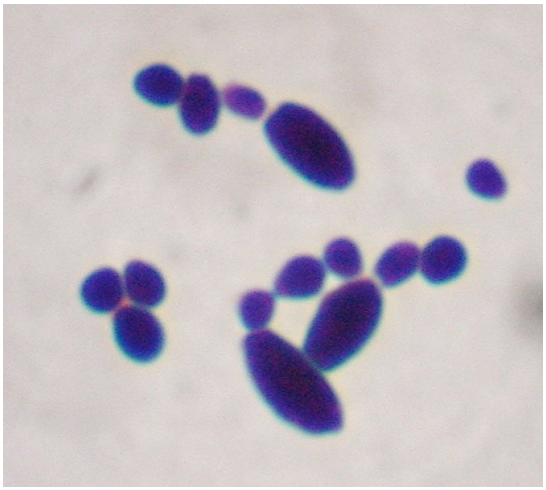


Candida albicans colonies have a buttery-like consistency on mycobiotic agar. Microscopically, *Candida* is composed principally of yeast cells, with occasional strings of unseparated budding yeast cells (pseudohyphae), and short structures resembling hyphae.



Malassezia pachydermatis (also called *Pityrosporum*) is a very common cause of otitis externa in dogs, especially those with heavy, floppy ears. We don't often culture it in our clinical laboratory because it is so easy to diagnose from clinical specimens. Ears infected with this organism have a characteristic bad smell. Microscopically, the organisms are oval or shaped like snowshoes or bowling pins. Somewhat surprisingly, there is rarely an inflammatory reaction in these cases, although a lot of brown discharge is produced that contains yeast organisms, squamous epithelial cells, and cerumen.

Other yeasts, like *Saccharomyces* -- worshipped by many veterinary medical students for its role in the production of beer -- are non-pathogenic. They produce white to tan colonies that have a smooth, buttery appearance. Microscopically, they consist of single or budding yeasts. Prepare a Gram stain of *Saccharomyces* and compare its microscopic morphology to that of



DIMORPHIC FUNGI

This class of fungi is characterized by two forms of growth:

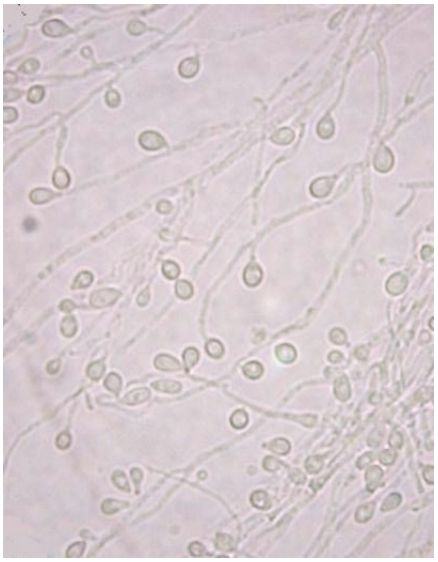
1. Growth as a mold with septate hyphae in their natural reservoir (e.g.soil) or when incubated at 25° C. on conventional fungal media (Sabouraud dextrose or potato dextrose agars).
2. Growth as a yeast in the tissues of an animal/person or when incubated at 37° on enriched media (Brain heart infusion agar).

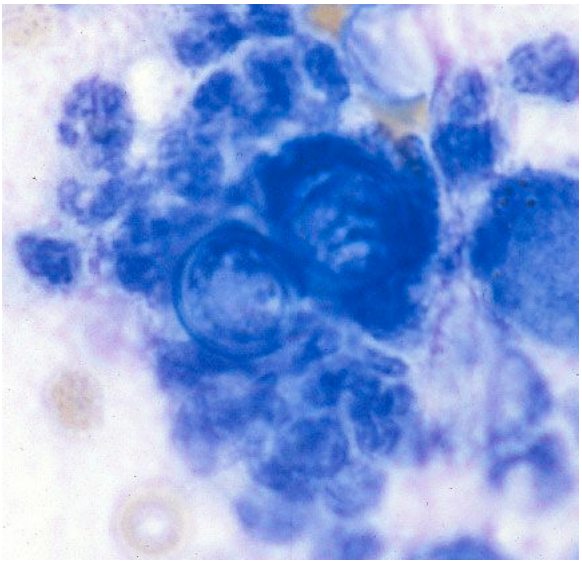
Compare the macroscopic colonial appearances of the yeast and mold forms of the organisms. The yeast forms have more of a moist appearance than the mold forms. The distinction is not always obvious, however, as the mold forms of these pathogens do not usually produce abundant aerial hyphae and therefore may lack the fuzzy appearance of common molds. In addition, conversion to the yeast form is often incomplete, resulting in cultures that have characteristics of both the mold and yeast forms of the organism. We will only provide the mold form of *C. immitis* because it is rather difficult to induce the yeast form of *Coccidioides immitis* in the laboratory because it requires increased levels of CO_2.

Although the colonial morphology of the dimorphs is rather nondescript, the microscopic appearance of these organisms, especially in clinical specimens, is much more useful in identifying them. The following pages include descriptions of the microscopic appearance of these dimorphic organisms.

Blastomyces dermatitidis

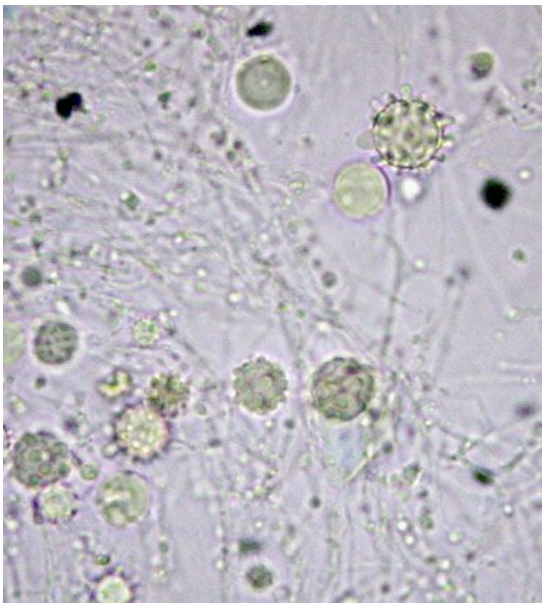
The **mold form** of *Blastomyces dermatitidis* produces single **"lollipop" conidia** directly from the septate hyphae or delicate conidiophores. The **yeast forms** from clinical specimens are **big, broad-based budding yeast cells with thick, double walls.**

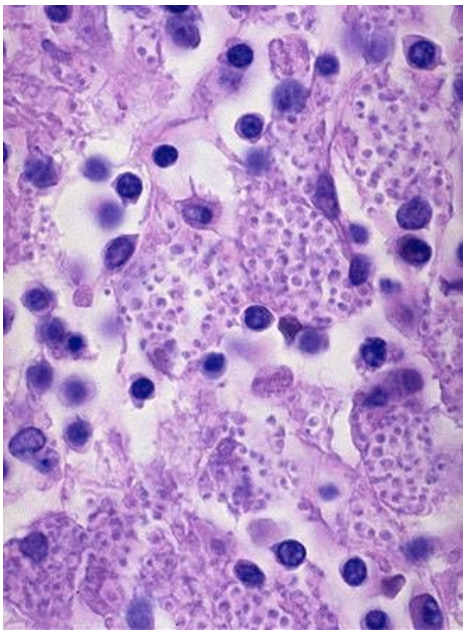




Histoplasma capsulatum

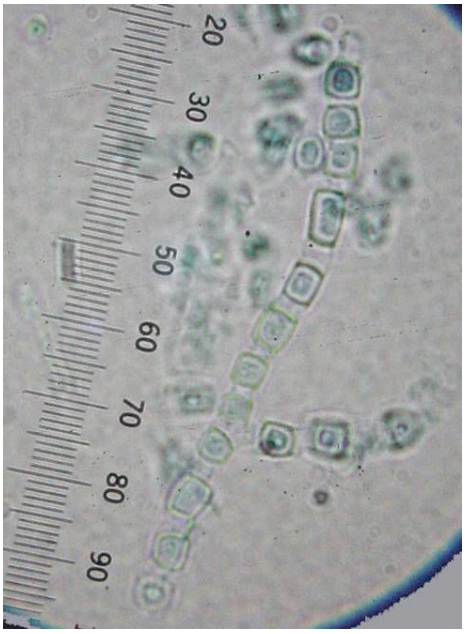
The **mold form** of *Histoplasma capsulatum* produces spiny, **tuberculate macroconidia** that form on narrow conidiophores. They resemble the steering wheel of an old-fashioned ship like the Mayflower or an expensive yacht. Microconidia are rare. The **yeast forms** seen in clinical specimens are small, single-celled or budding **yeasts present within macrophages**.

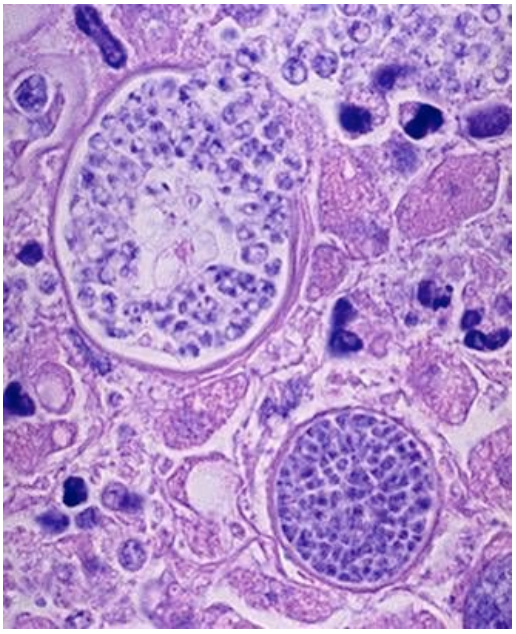




Coccidioides immitis

The **mold form** of *Coccidioides immitis* produces **barrel-shaped arthrospores** which are formed by fragmentation of the hyphae. The arthrospores are the **infectious form** of the organism. The **yeast forms** seen in clinical specimens are **huge spherules that contain numerous small endospores**.





Sporothrix schenckii

The **mold form** of *Sporothrix schenckii* produces **thin, branching hyphae with delicate conidiopheres that bear clusters of small teardrop-shaped conidia** at their tips. They resemble flowers on delicate stems. The **yeast forms** seen in clinical specimens are **cigar-shaped cells** that are also known as “cigar bodies.” The inflammatory reaction sometimes seen in the body is called an asteroid body.

DIMORPHIC FUNGI

Microsporum canis

The septate hyphae of *Microsporum canis* produce **large, thick-walled, multi-septate, canoe-shaped macroconidia**. Single macroconidia are borne directly from the hyphae or by attachment to a specialized cell. Microconidiospores are sparse if present at all.

Microsporum gypseum

The septate hyphae of *Microsporum gypseum* produce large **macroconidia** that are similar to those of *Microsporum canis* except that they are **rowboat-shaped with more rounded ends**. Microconidia are conspicuously absent.

***Trichophyton* sp.**

The septate hyphae of *Trichophyton* produce numerous small microconidia that form along the sides of the hyphae in clusters. Macroconidia are similar to those of *Microsporum* sp. except that they are cigar-shaped and have thinner, smoother walls. They are also quite rare, so don't spend a lot of time looking for them. If you are lucky enough to find one, let the rest of us know!