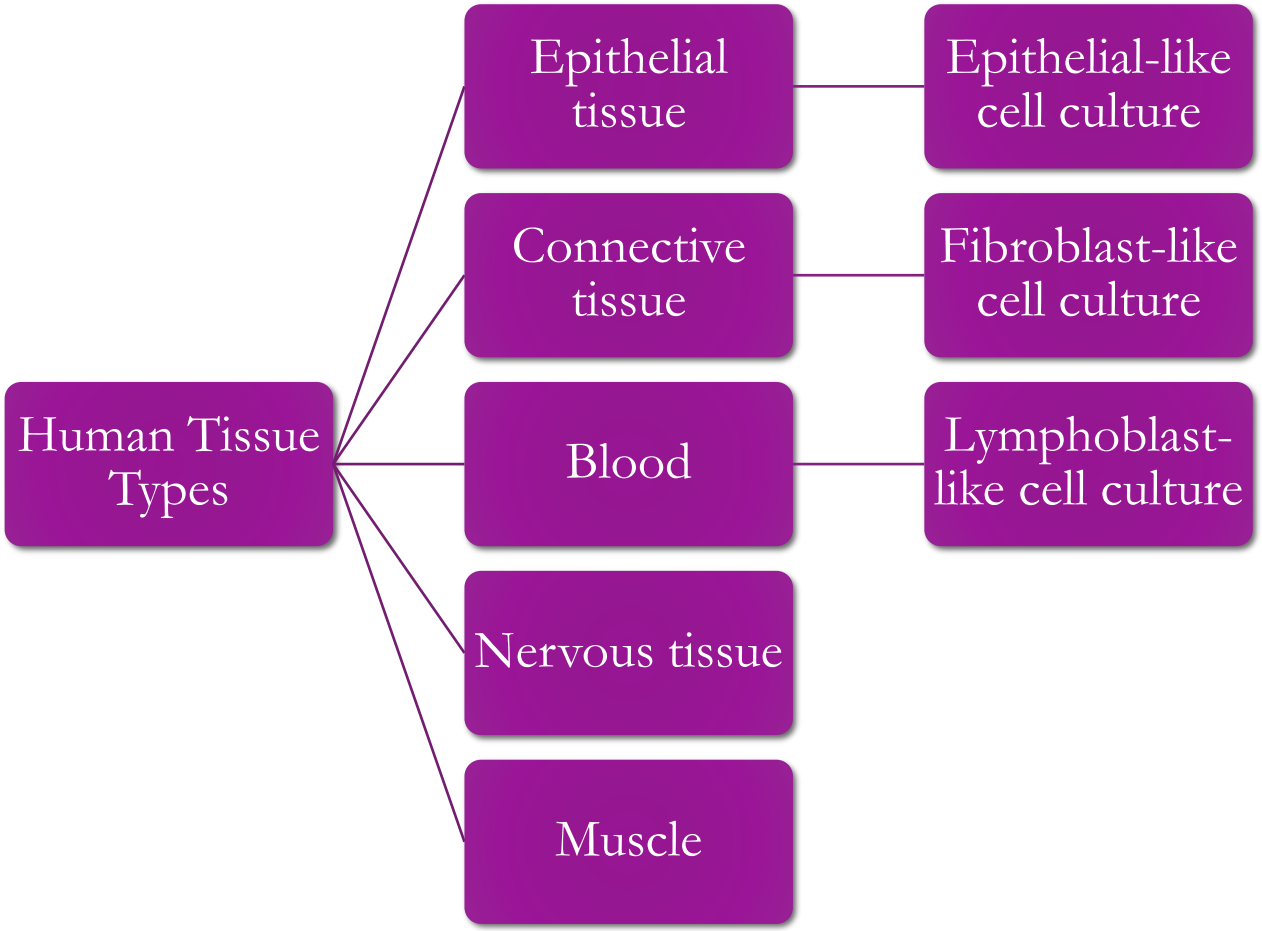


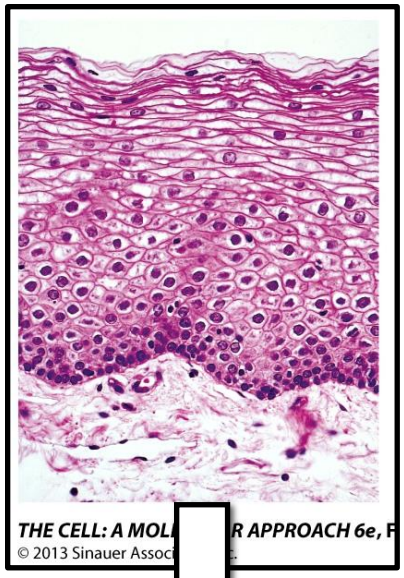
Cells in Culture

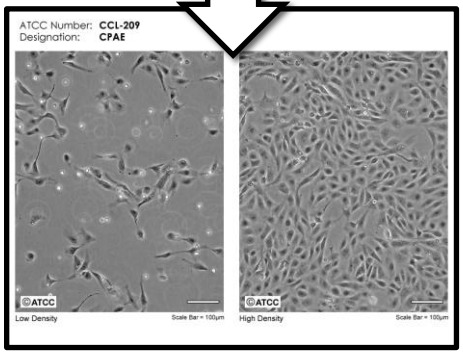
characterization and expectations

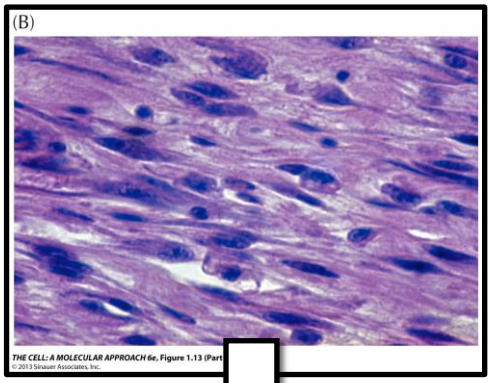
TYPES OF CELLS IN CULTURE

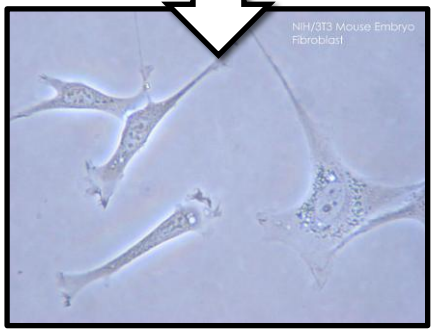


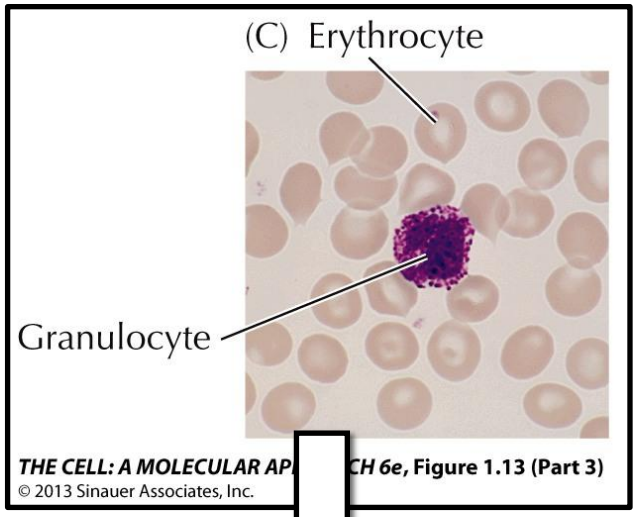
TYPES OF CELLS IN CULTURE

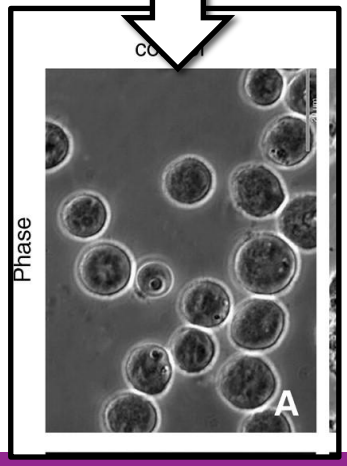


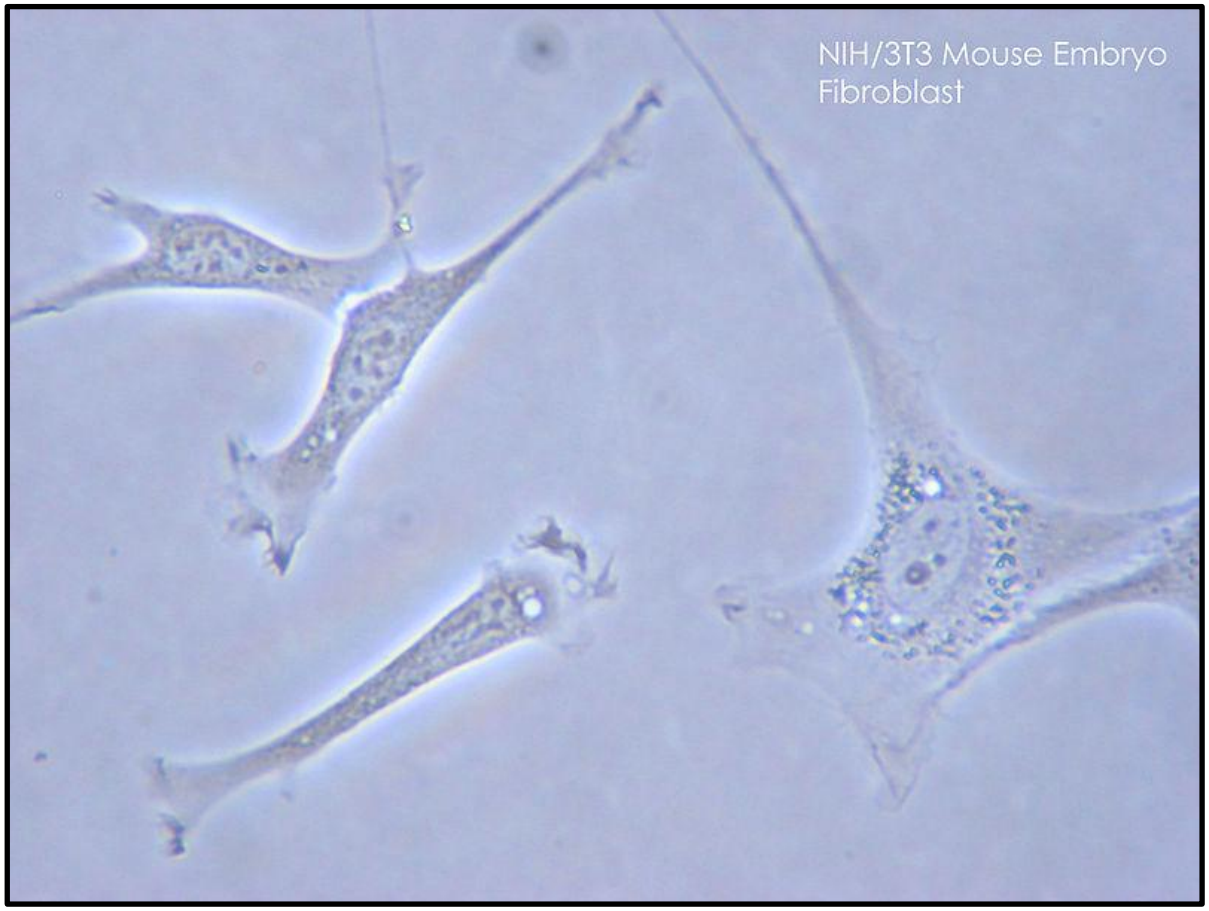




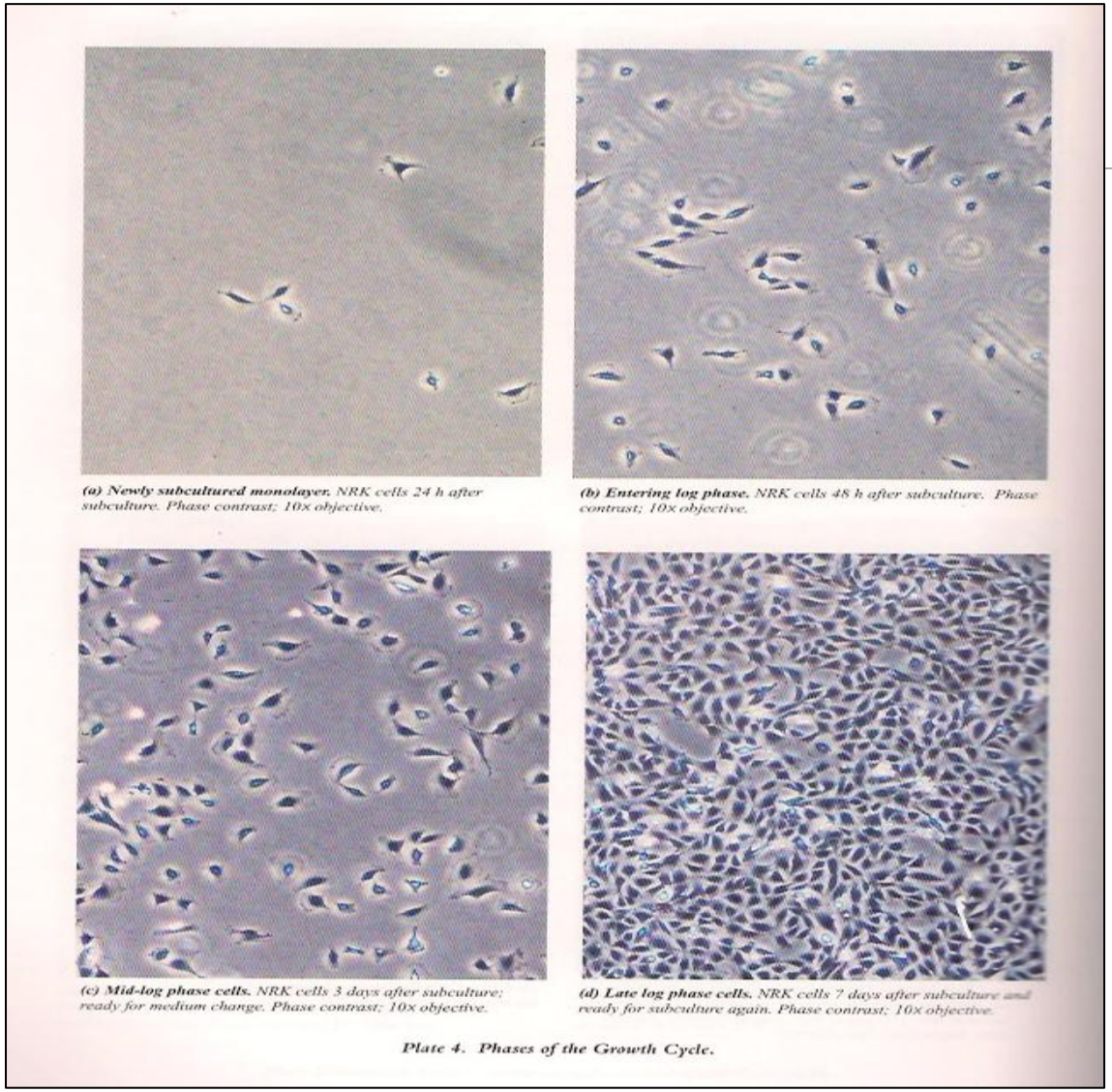







NIH/3T3 Mouse Embryo
Fibroblast

Cell Density and Morphology



(a) Newly subcultured monolayer. *NRK cells 24 h after subculture. Phase contrast; 10× objective.*

(b) Entering log phase. *NRK cells 48 h after subculture. Phase contrast; 10× objective.*

(c) Mid-log phase cells. *NRK cells 3 days after subculture; ready for medium change. Phase contrast; 10× objective.*

(d) Late log phase cells. *NRK cells 7 days after subculture and ready for subculture again. Phase contrast; 10× objective.*

Plate 4. Phases of the Growth Cycle.

At low cell density epithelial cells might resemble fibroblasts.

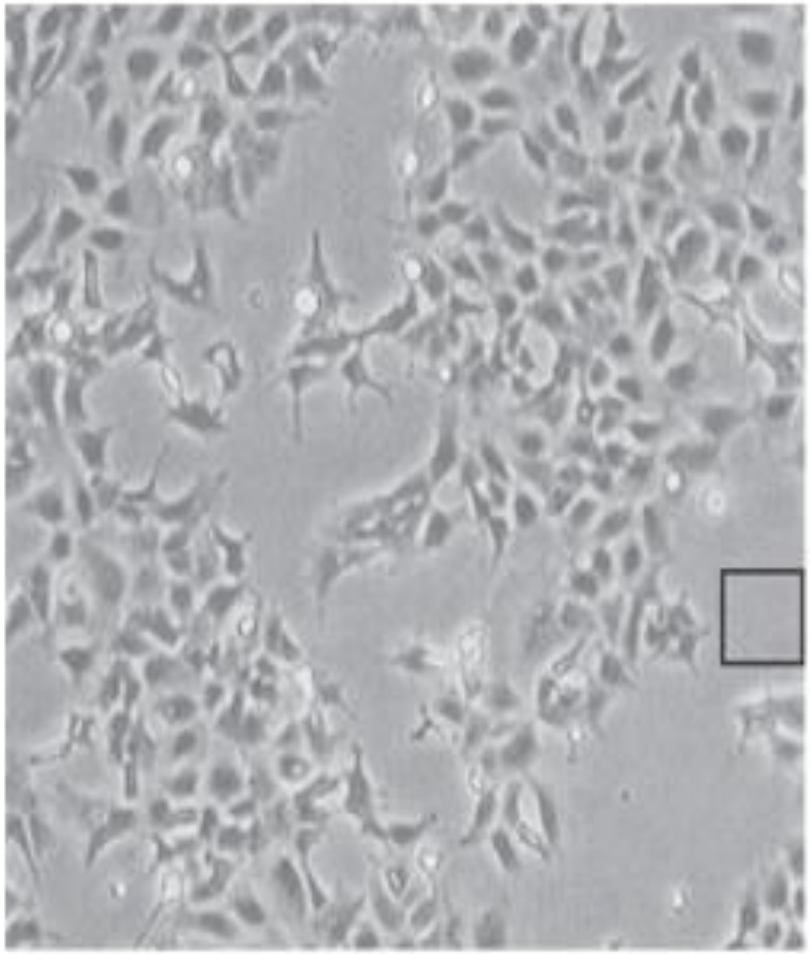
The correct morphology will be most distinguishable in a confluent dish.

Identifying Contamination



OR??

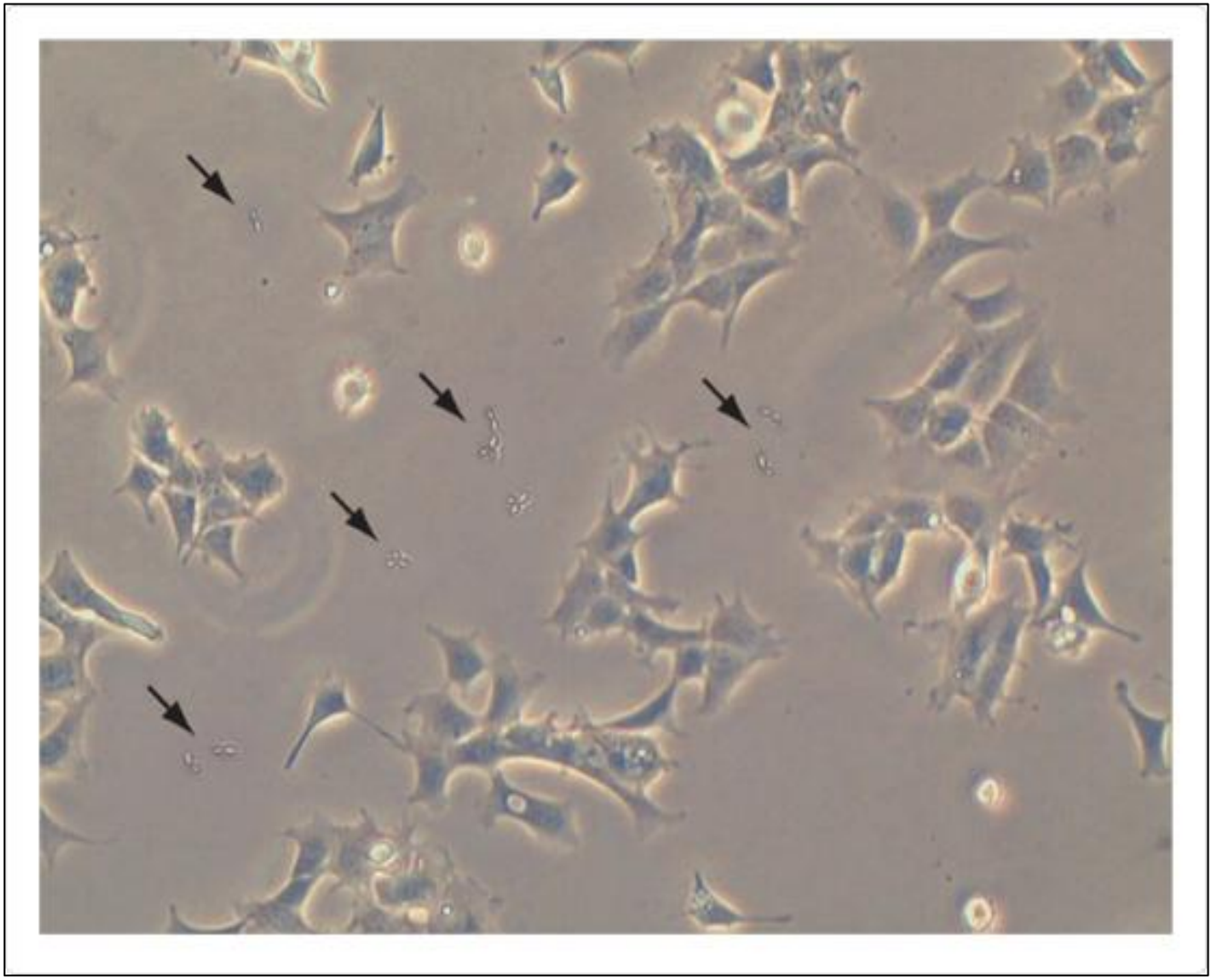






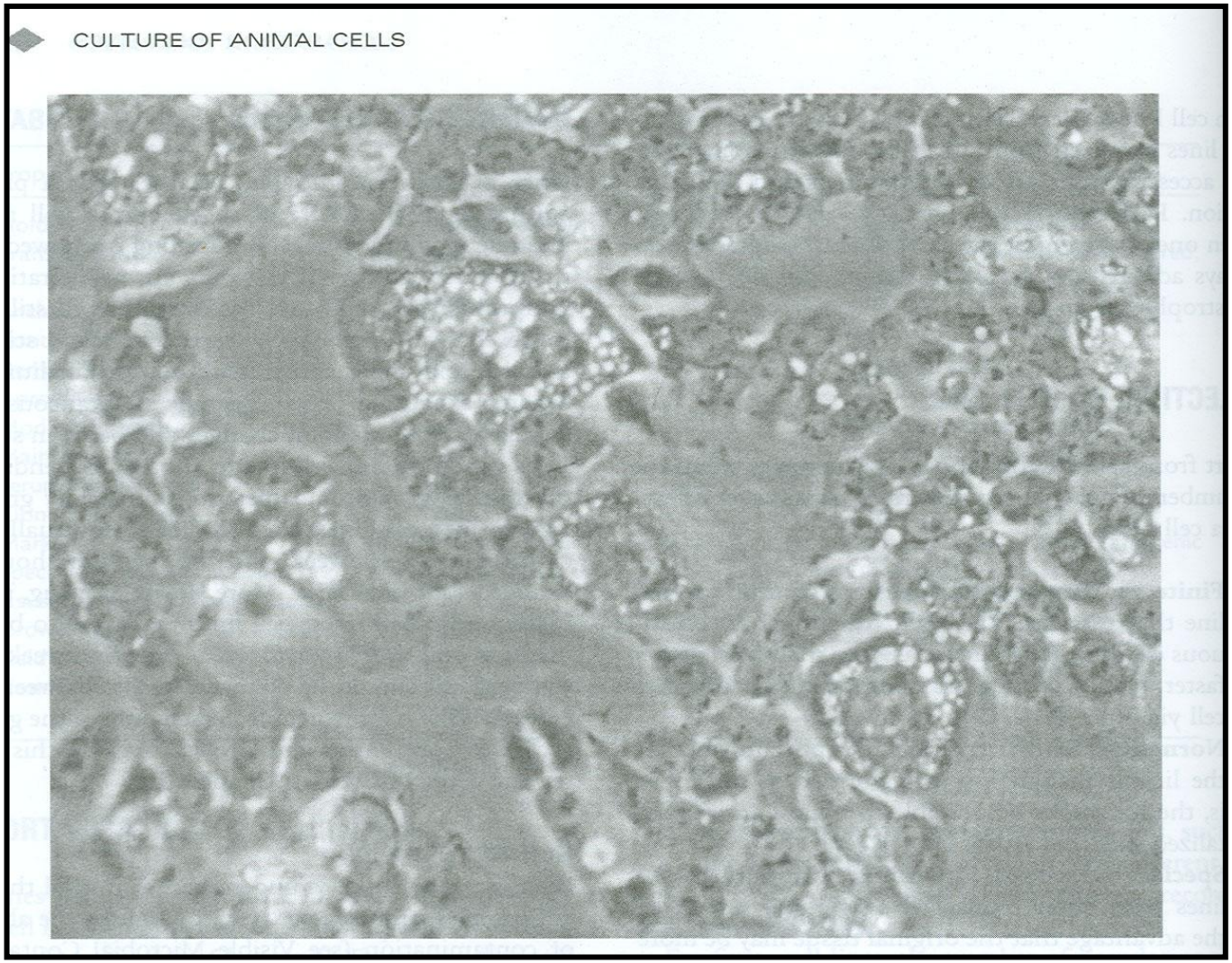
Human cells are approximately 10μm, while bacteria are 1μm.

http://www.lifetechnologies.com/us/en/home/references/gibco-cell-culture-basics/biological-contamination.html



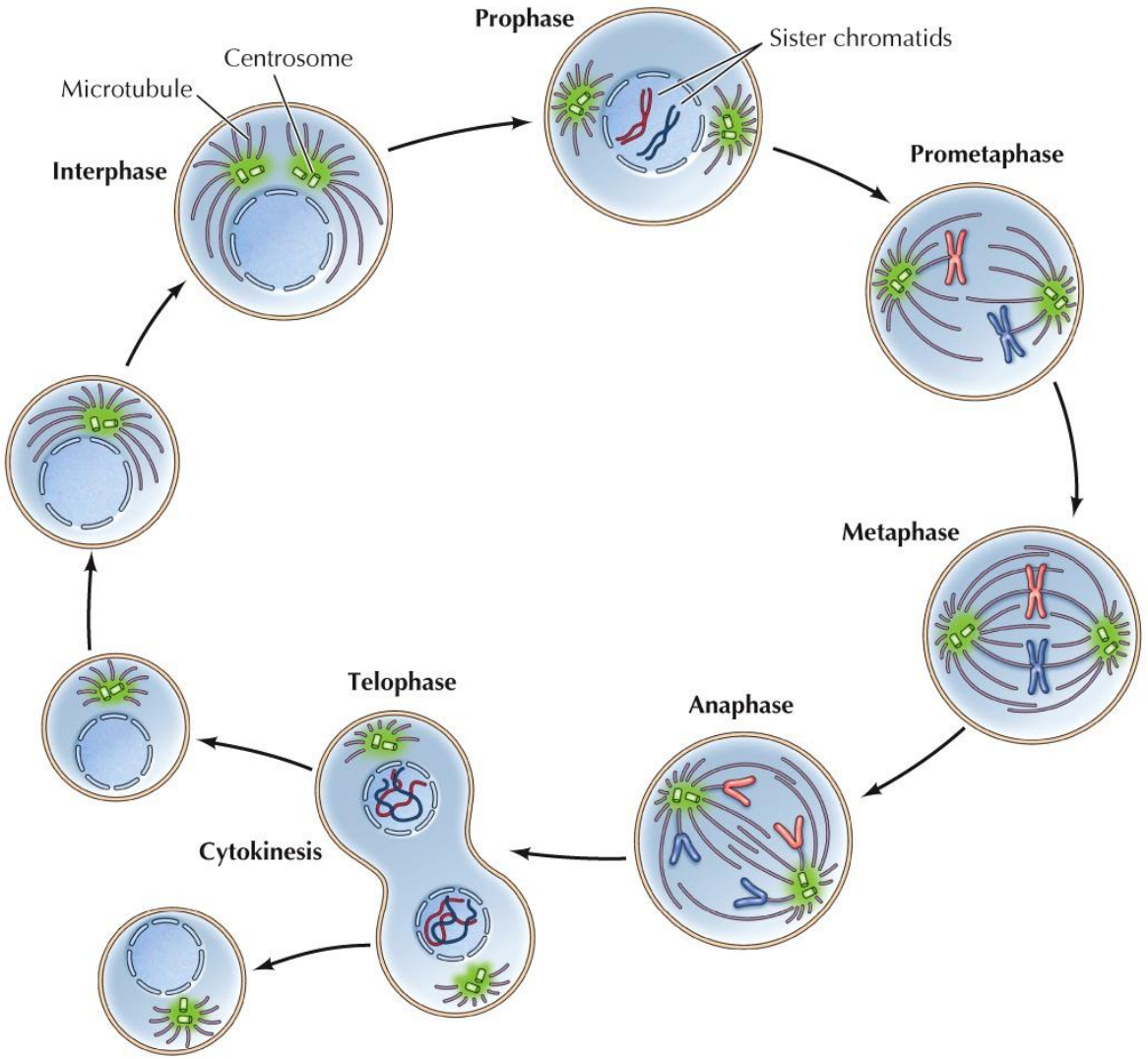
Yeast are larger than bacteria. Arrangement and characteristic budding can be used for identification

http://www.lifetechnologies.com/us/en/home/references/gibco-cell-culture-basics/biological-contamination.html



An overall decline in cell health can indicate contamination that may not be visible under phase contrast microscopy.

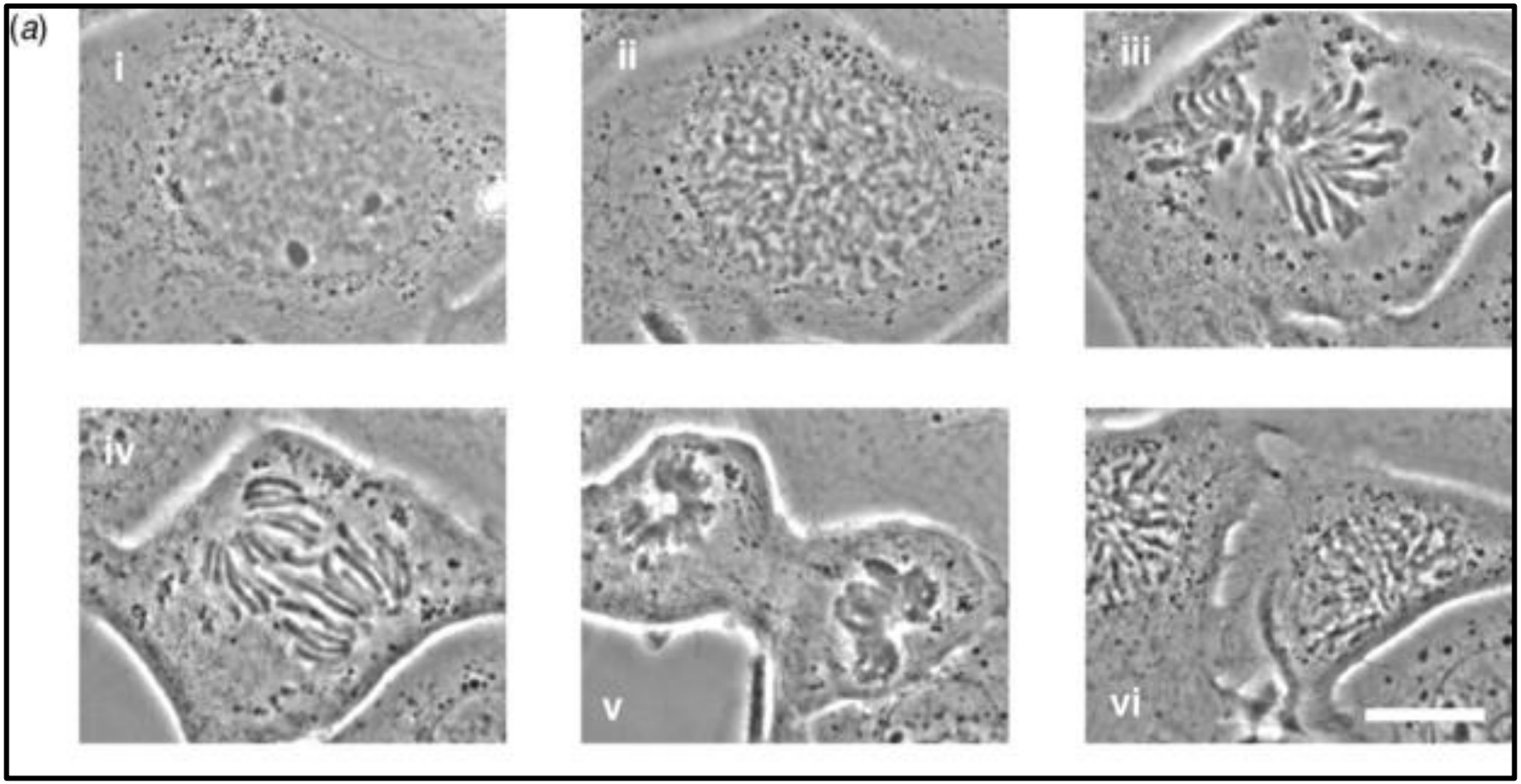
Identifying Cells in Mitosis



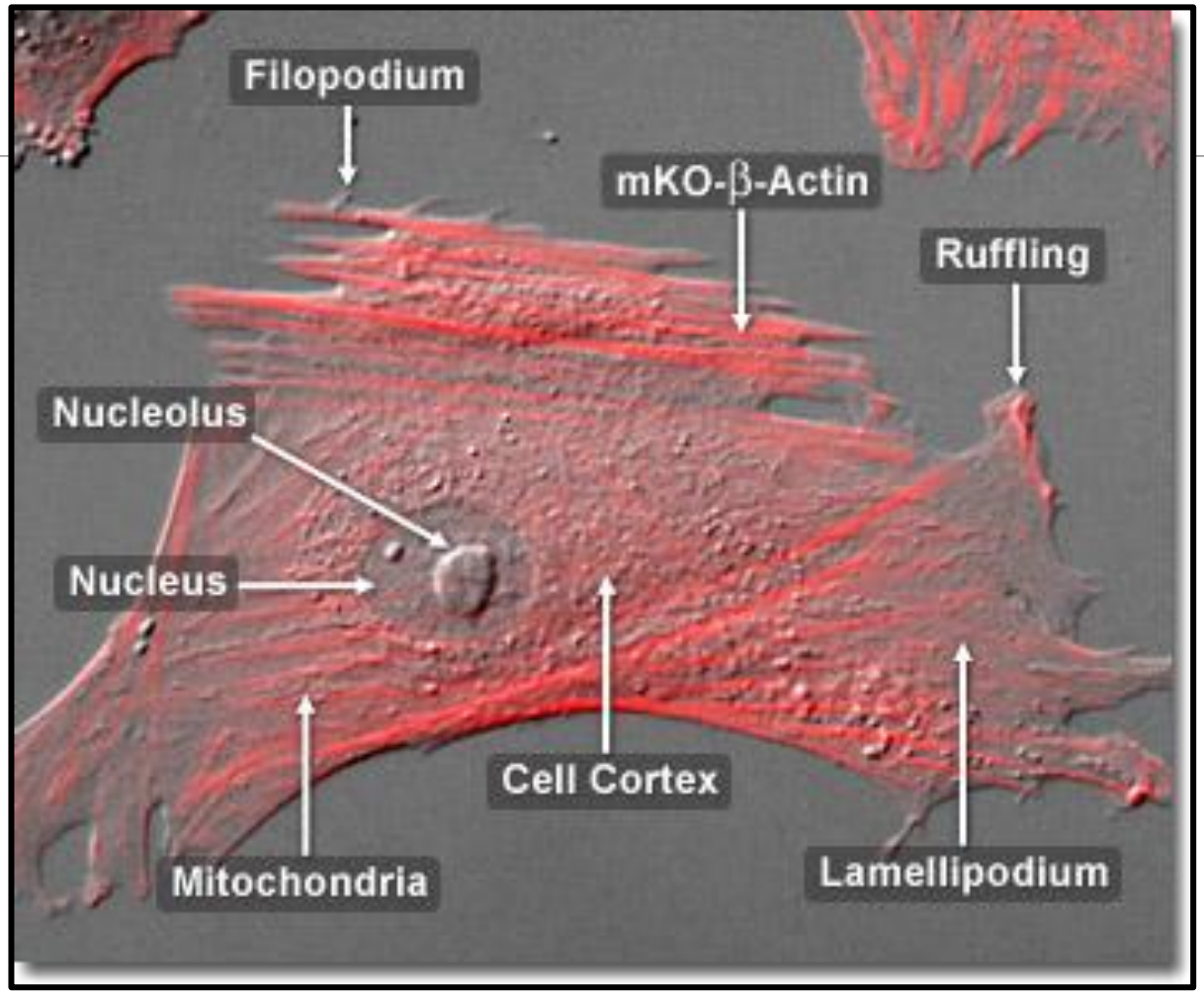
***THE CELL: A MOLECULAR APPROACH 6e*, Figure 16.21**

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Identifying Cells in Mitosis



Identifying Elements of Motility



http://olympusmicro.com/moviegallery/confocal/folumkoactin/index.html

<http://www.microscopyu.com/moviegallery/livecellimaging/3t3/index.html>

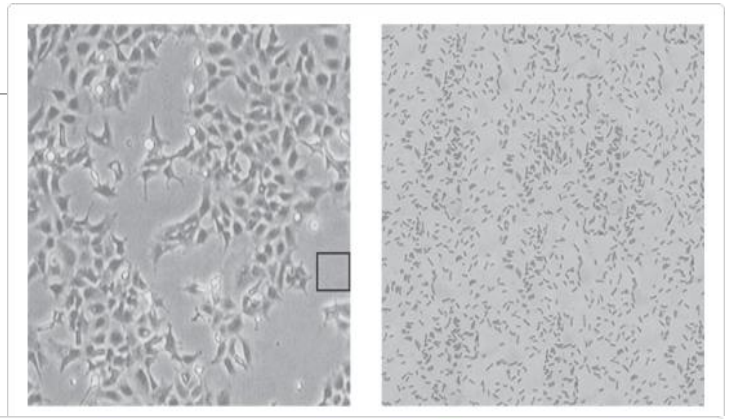
Identifying Contamination by Poor Aseptic Handling or Storage

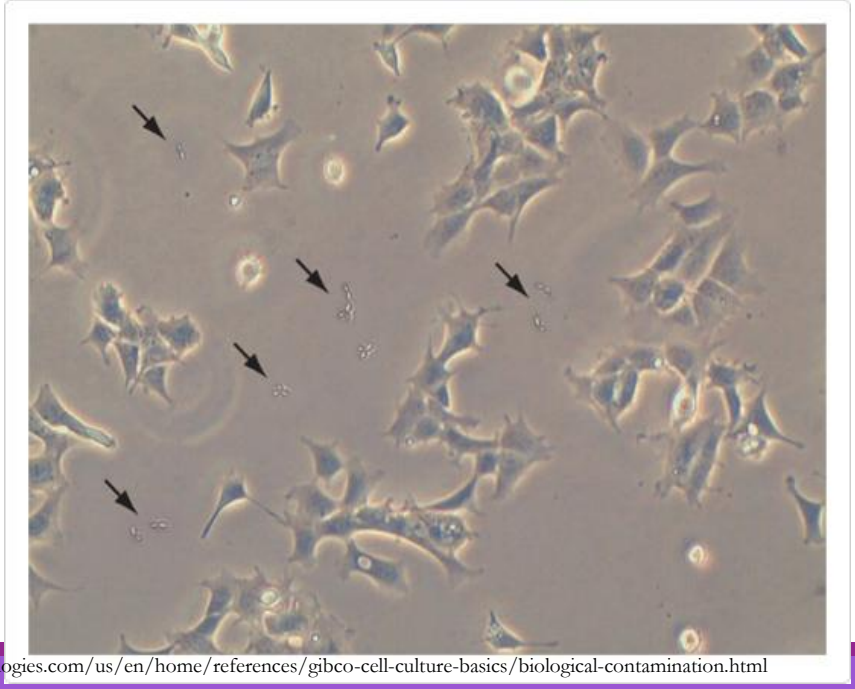
1. **Bacteria**
   - Cloudy or floculated media, drop in pH
   - Distinguish cocci vs. rods

2. **Yeast**
   - Cloudy media with no drop in pH
   - Often can identify budding yeast

3. **Mold**
   - Turbid media, increased pH
   - Filamentous hyphae

4. **Viral**
   - Very difficult to ID given size
   - If present, potentially dangerous

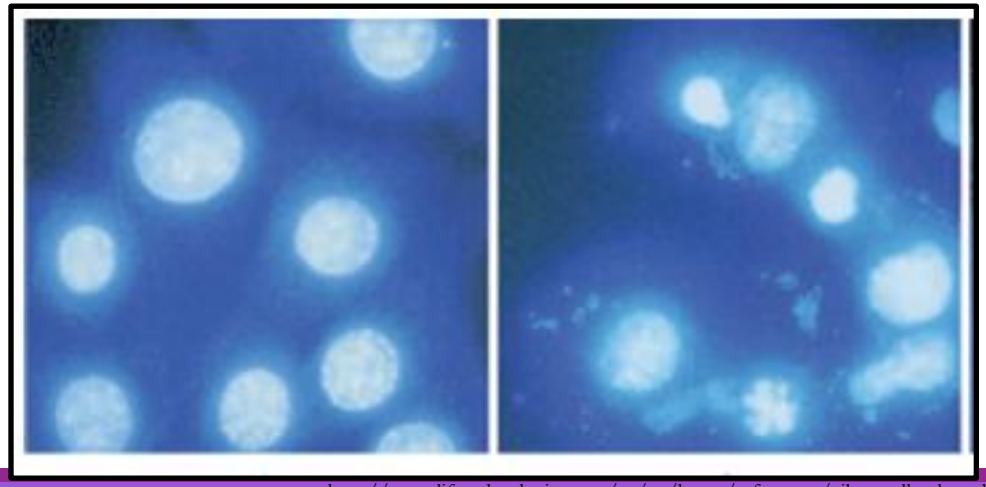




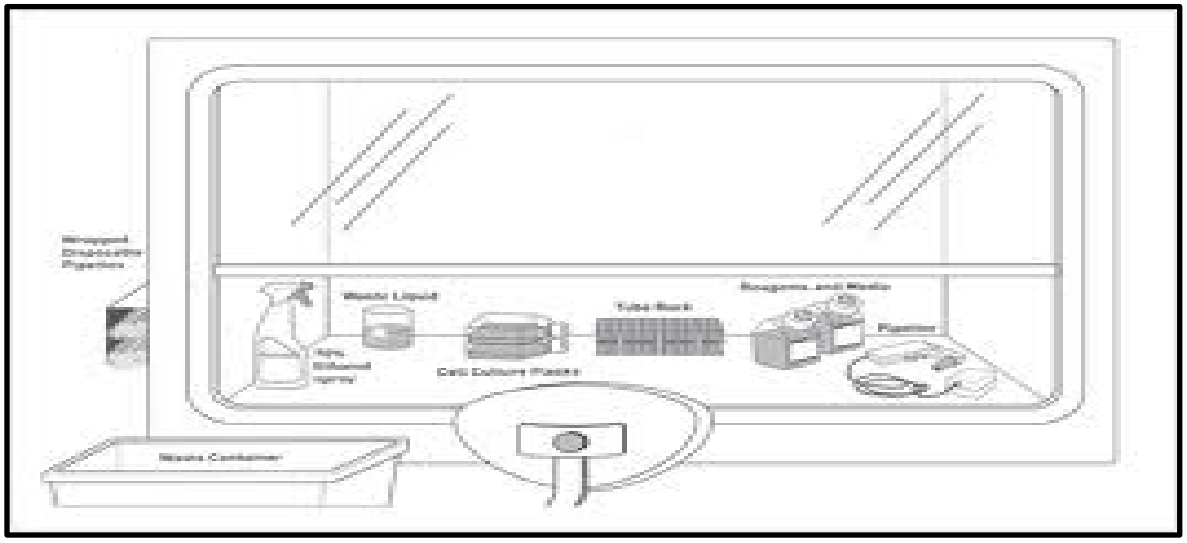
http://www.lifetechnologies.com/us/en/home/references/gibco-cell-culture-basics/biological-contamination.html

Identifying Contamination by Poor Aseptic Handling or Storage

5. **Mycoplasma**
   - Bacterial contaminant, but because of their extremely small size (<1um) they are very difficult to detect
   - Can persist in cultures not causing cell death be causing changes in metabolism, cell growth, behavior and even chromosomal aberrations of the host cells
   - Cultures should be tested periodically using fluorescent staining of DNA, PCR, ELISA or alternate methods



http://www.lifetechnologies.com/us/en/home/references/gibco-cell-culture-basics/biological-contamination.html



Aseptic Technique

AND HOW TO AVOID CONTAMINATION

Aseptic Technique (*n*)

Task performed in a sterile environment in order to avoid introducing outside contamination

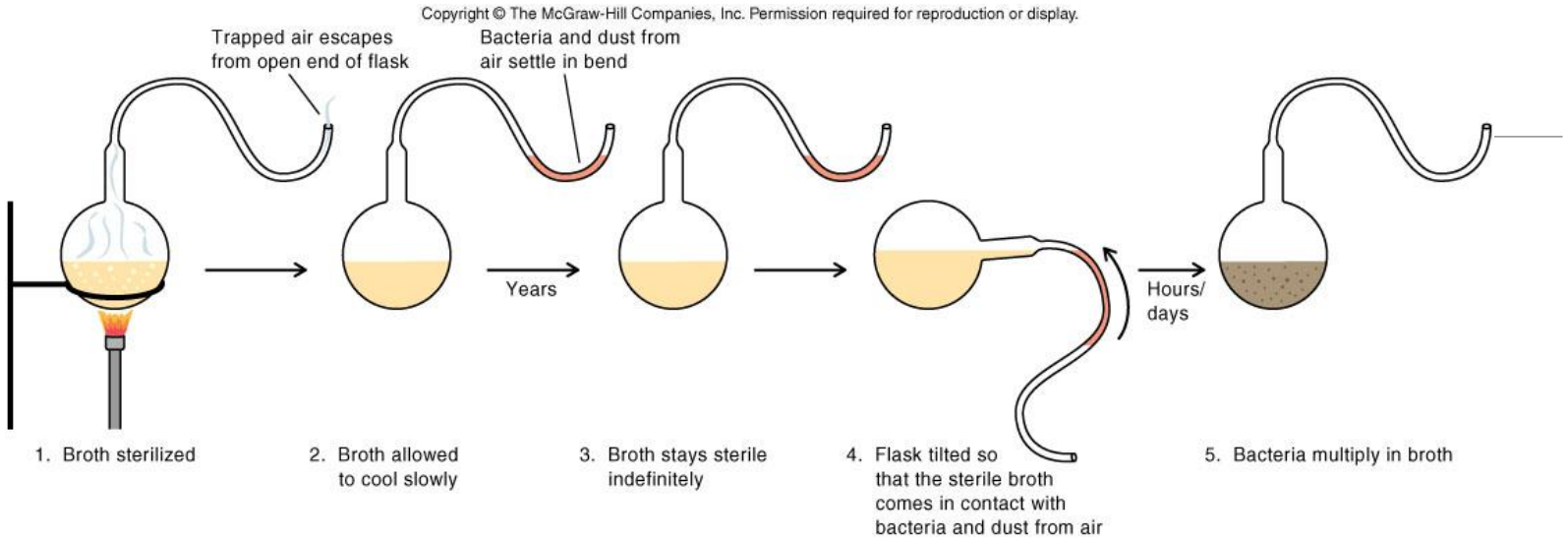
Where do organisms come from?

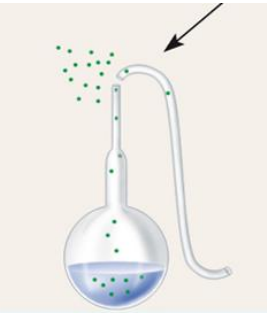
Two different ideas had to be hashed out:

- Biogenesis: life comes from pre-existing life
- Spontaneous generation (abiogenesis): life can come from non-living sources

Disproving Spontaneous Generation

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Neck on second sterile flask is broken; growth occurs.

(+) Control

Common Contaminants of cell culture:

Bacteria

Mycoplasma

Yeast

Fungal spores

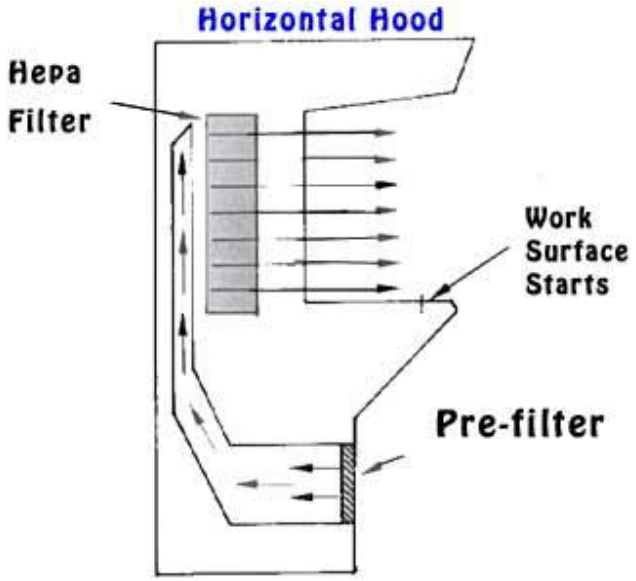
OBJECTIVES OF ASEPTIC TECHNIQUE

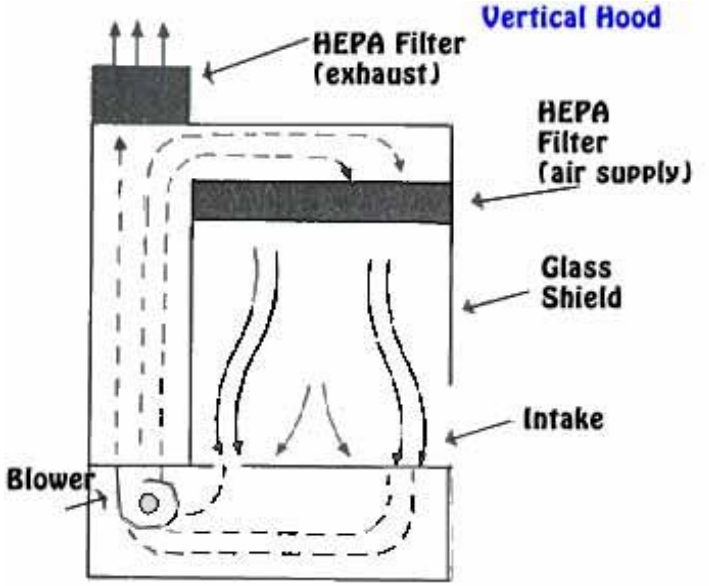
1. **Minimize risk of contamination**
   - From the operator, atmosphere, work surfaces, solutions, and other equipment.
   - Contamination can be minor and only damage one or two cultures, or it can be a catastrophe and wipe out an entire stock!

2. **Reduce risk to personnel**
   - Minimize the risk of exposing personnel to any potentially biohazardous materials being handled.

ELEMENTS OF AN ASEPTIC ENVIRONMENT

1. **Laminar flow**
   - Establishes a "clean-air space." Not a sterile environment
   - Two main types: horizontal or vertical
   - The work area must be kept clear, all spills cleaned immediately, and sterility protocols adhered to strictly
   - Anything INSIDE the hood must be CLEAN. Anything OUTSIDE the hood is assumed DIRTY.
     → 70% ethanol is your friend

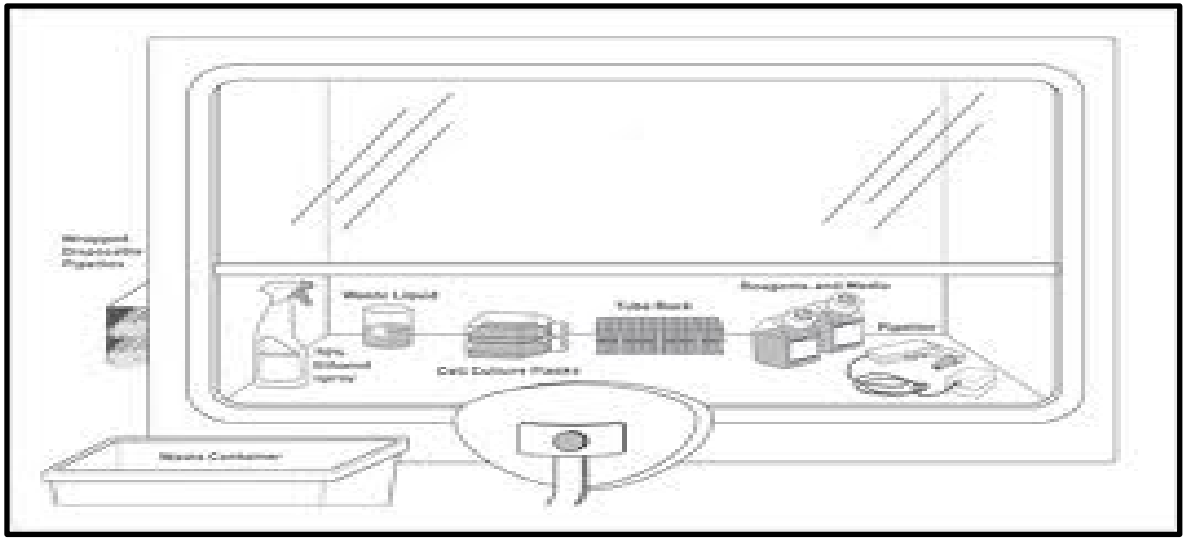




ELEMENTS OF AN ASEPTIC ENVIRONMENT

2. **Quiet area**
   - Minimize traffic, dust, and movement in the hood room.

3. **Work surface**
   - It is essential to keep your work surface clean and tidy
   - Only the materials you need for the current procedure should be in the hood.
   - Arrange your materials around the periphery



ELEMENTS OF AN ASEPTIC ENVIRONMENT

4. **Personal hygiene**
   - Wash your hands before and after working in the hood.
   - Always wear gloves during all procedures.
   - Long hair should be tied back. Lab coats are recommended but not required. Wear a face mask if you have a cold (or let your lab partners handle the aseptic procedures).

5. **Incubators**
   - A dangerously microbe friendly place where we have to maintain our sterile cultures!

STERILE HANDLING

1. **Swabbing/Spraying**
   - Swab all work surfaces and equipment with 70% alcohol before work
   - Gloved hands and all objects should be sprayed with liberal amounts of 70% alcohol every time they enter the laminar hood.
   - Clean up any spills immediately, using 70% alcohol

2. **Capping**
   - All reagents/media/cultures should be kept capped when not in immediate use.

3. **Handling bottles and flasks**
   - Do not pass you hands or any equipment over an open bottle or flask.
   - Sterile bottles and flasks should only be opened in the hood.
   - Minimize your touch. Don't use a bear claw.

4. **Pipetting**
   - All liquids should be transferred via a pipet.



ANTIMICROBIAL PRODUCTS



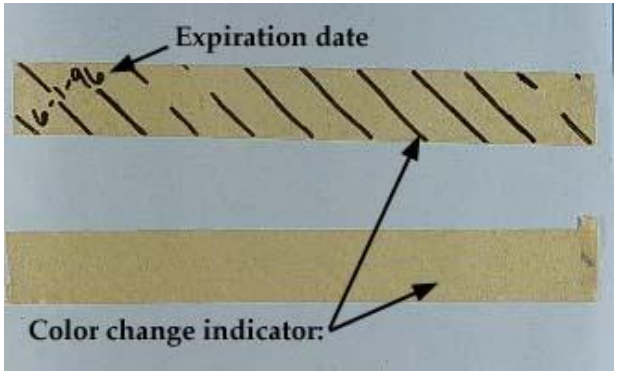




ANTIMICROBIAL PRODUCTS







Protocol 1.1. Aseptic technique for cell culture in laminar hood (bench top 408)

Starting up.

1. Put on gloves.

2. Turn on fluorescent light and fan.

3. Clear the surface area (pipets, etc).

4. Spray all areas (working surface, and all sides) with **BDD**, then wait three minutes. Yes, 3 minutes.

5. Wipe area down with kimwipes, toss in garbage.

6. Spray gloves and then all surfaces with **70% EtOH**. Wipe down all working surfaces.

AT THIS POINT THE HOOD IS CONSIDERED STERILE. ANYTHING GOING INTO THE HOOD FROM THIS POINT MUST BE WASHED IN 70% EtOH.

PROCEED WITH SCIENCE AT YOUR DISCRETION.

Finishing up.

1. Properly dispose or store all materials such that the hood is clear of any clutter. Wipe up any spills with a kimwipe, toss in garbage.

2. If media or cells were aspirated, use suction to run a small amount of BDD then 70% EtOH through tubing.

3. Spray all surfaces with **70% EtOH** and then wipe down.

4. Spray all surfaces with **BDD**. Wait three minutes. Wipe down.

5. If you are the last group using the hood then you must empty the waste container (pour down the sink with running water) and turn off the vacuum pump. You must also turn off the fluorescent light.

6. Remove gloves and wash hands.

Protocol 1.2. Aseptic technique for cell culture in laminar hood (Rm 408 A and B)

Starting up.

1. Turn on **UV light** with sash DOWN. Wait 10 minutes. Turn off UV light.

2. Put on gloves.

3. Lift sash to turn on fluorescent light and fan.

4. Spray gloves and then all surfaces with **70% EtOH**. Clear the surface area (pipets, etc.). Wipe down all working surfaces.

AT THIS POINT THE HOOD IS CONSIDERED STERILE. ANYTHING GOING INTO THE HOOD FROM THIS POINT MUST BE WASHED IN 70% EtOH.

PROCEED WITH SCIENCE AT YOUR DISCRETION.

Finishing up.

1. Properly dispose or store all materials such that the hood is clear of any clutter. Wipe up any spills with a kimwipe, toss in garbage.

2. If media or cells were aspirated, use suction to run a small amount of BDD then **70% EtOH** through tubing.

3. Spray all surfaces, including the UV bulb, with 70% EtOH and then wipe down.

4. Close sash. Turn on **UV light** and wait 10 minutes. Turn off UV light.

5. If you are the last group using the hood then you must empty the waste container (pour down the sink with running water) and turn off the vacuum pump.

6. Remove gloves and wash hands.