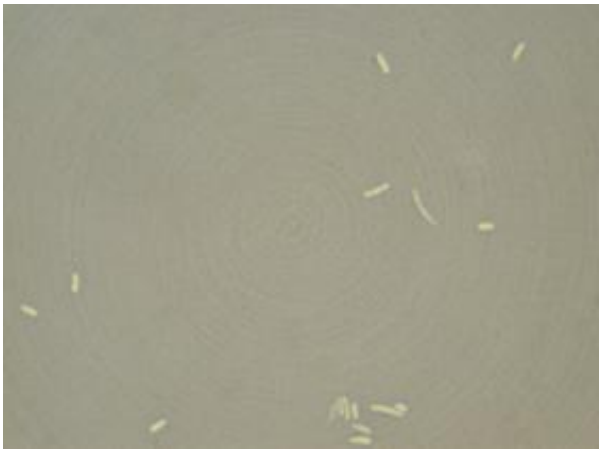
Negative Stain

Negative stains are even simpler than simple stains because you do not have to make a smear. A drop of cells is spread on a slide and viewed without fixation. The stain is a suspension of carbon, found in India ink or nigrosin. The carbon particles are negatively-charged, as is the cell membrane. The background looks black or sepia colored and the cells remain clear, since they repel the dye. Some positively charged inclusion bodies such as sulfur may stain. This stain gives accurate information on cell morphology and capsule presence because the cells are not fixed. Cell size appears slightly larger because any extracellular coatings or secretions on the outside of the cell membrane also do not stain. Negative stains are useful for rapid determination of the presence of *Cryptococcus neformans,* the causative agent of cryptococcisis, in cerebral spinal fluid. This technique is also used when you stain for endospores and capsules.



Enterobacter aerogenes

Materials

- Nigrosin dye
- Assorted cultures

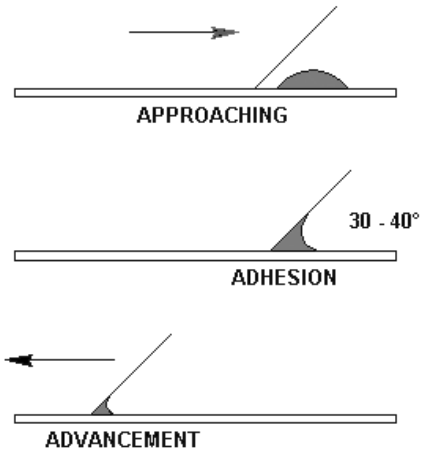
General Considerations

Just as in preparing a smear, you only need a small amount of organism. If you have too many organisms, you won't be able to see the morphology of individual cells. It is also important not use too much nigrosin. If it is too thick, the background will have a cracked appearance similar to mud puddles drying in the sun. You want to get a light film. Your instructor will demonstrate this technique for you.

Negative Staining Procedure

1. Label your slide. If you are working from a broth culture, place a loop-full of organisms about three fourths of the way on the left side of the slide. If you are working from a plate culture, add a drop of sterile water to the slide and dilute your organism in the drop without spreading the drop.
2. Put one or two drops of nigrosin on another slide. Use your sterilized loop to pick up a loop-full of nigrosin. Carefully mix it in with the drop of cells, without spreading the drop too much.
3. Hold the right end of the slide in your right-hand; with your left–hand take another slide at a 45° or less angle to the first slide, just past your nigrosin/cell drop.

4. Scoot the angled slide back along the surface of the first slide till it **just** touches the drop of nigrosin and cells. Wait for capillary action to draw the liquid along the leading edge of the angled slide.
5. Push the angled slide across the surface of the flat slide. Most of the nigrosin should still be left on the original spot. Discard the slide in the disinfectant bucket.
6. Set the stained slide aside to air dry before observing it under oil immersion. Be sure to start examining your slide in the area with the faintest gray background.
7. Record your observations in your lab book.
8. Discard your used stained slide in the disinfectant bucket.



Special Note

Nigrosin comes off the slide and onto your oil immersion lens very easily. Be sure to thoroughly clean your oil lens when you are finished. Then clean it again. Once it dries on the lens it is very difficult to remove and will impair your ability (and the other micro students using that scope) to see clearly out of the lens.