Research projects in this course tend to involve principally three types of microscopy: phase-contrast, fluorescence, and scanning electron. This introductory demonstration is intended to exhibit for you the features of microorganisms and microbial populations that are revealed by each method so that you will be able to judge whether and to what extent each method might be useful to you during the course.

Phase-contrast microscopy

Specimen: wet mount of living or fixed, but unstained microorganisms.

Features discernible: true size; shape; motility; orientation of adhesive bacteria to substrata; some appendages, but not prokaryotic flagella. In eukaryotes, also: color; flagella; shape, size, location, and movement of organelles such as nuclei, vacuoles, mitochondria, chloroplasts.

Other uses: Total cell counts employing a counting chamber.
Estimation of respiring bacteria in a natural population by addition of tetrazolium.

Phase-contrast microscopes are available in the class laboratory (Loeb 208); the phase-contrast microscope in Loeb 205 is available for photomicroscopy. Each of these microscopes is also suitable for dark-field microscopy.

Fluorescence microscopy

Specimens: wet mounts of auto-fluorescent cells or of stained cells.

a) Auto-fluorescence of chlorophylls can be observed within photosynthetic cells, and of factor 420 in methanogens. The excitation wavelengths and the wavelengths emitted are different for these two types of fluorescence; they must be observed with different sets of filters allowing passage of the appropriate wavelengths from light source (a Xenon lamp) to specimen, and from specimen to viewer.

b) Acridine orange stain fluorescence: AO selectively stains nucleic acids. AO-RNA fluoresces red-orange within cells, whereas AO-DNA fluoresces green. The AODC (acridine orange direct count) method allows simultaneously:
concentration of cells in a dilute sample,
relatively specific staining of bacteria and not of inanimate particles that are otherwise difficult to distinguish from bacteria,
a color differential between actively growing (orange) and probably viable, but not growing (green) bacteria, and
a determination of the relative frequency of dividing cells and thereby a rough approximation of bacterial reproductive activity in the sample.
Filter preparation, and staining, collecting and mounting of cells will be demonstrated.

The microscope available in Loeb 205 provides simultaneous phase-contrast and epifluorescence optics, and photomicroscopy.

Specimen: fixed, dehydrated, critical point-dried in liquid CO_2, and Au·Pt sputter-coated.
The specimens used for this demonstration have been prepared in advance to save time for microscopy. Specimen preparation will be demonstrated when the need arises during the course.

Features discernible: cell surfaces, three-dimensional aspects of cell shape and of spatial relationships among microbes and between microbial cells and substrata to which they adhere.

The SEM available for use in the course is located in Lillie 208. Microscope time must be reserved in advance; there is a sign-up sheet on the door of L208.

Transmission electron microscopy

TEM of shadowed and of negatively-stained specimens is also available, but will not be demonstrated during this introduction. (Preparation of ultrathin sections will not be included in the course work.) The preparation of specimens and use of the microscope will be demonstrated as need arises.

The TEM available to this course is located in Loeb 212C. Microscope time must be reserved in advance; there is a sign-up sheet on the door of Lb212C.

References

A collection of relevant references is available in a folder in the drawer of the microscope desk in Loeb 205. Please use them locally and return them to the drawer (or make copies for your own use, if you want).