Bacterial Abundance

Objective

- Measure bacterial numbers and mass per unit volume.

- Note, we are not concerned with identification here.

Why do we want to know abundance?

- Allows determination of biomass pool size.

- Provides crude estimate of element fluxes.

- Helps to characterize dynamics of ecosystem.

Challenges with natural samples

- Low concentrations

Methods

- Dry and weigh (not with natural samples).

- Plate (or viable) count (Today).

- Direct count. (Thursday).

# Why do we want to measure bacterial concentration?

E.g., **Bacterial concentration** is 100 cells ml^{-1} or 100 fg C ml^{-1}
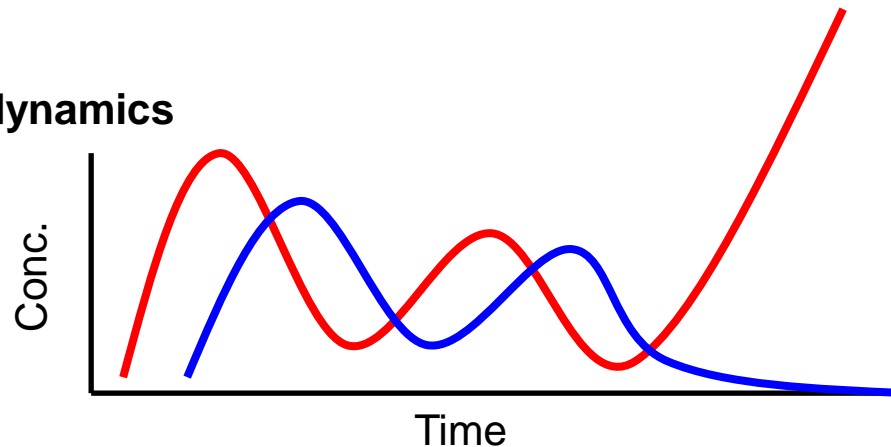
Estimate bacterial pool size

- Ocean: 10^9 cells l^{-1}

  20 fg C $cell^{-1}$ (20×10^{-15} g C $cell^{-1}$)

  1.37×10^{21} l $oceans^{-1}$

  } 27 Pg C $oceans^{-1}$

Atm: 850 Pg

Oceans
  Organic: 700 Pg
  Inorganic: 38,000 Pg

Crude estimate of element fluxes (x: bacterial biomass)

- Growth rate: $G = \mu x$; μ: specific growth rate

  Uptake rate: $U = \mu x/\varepsilon$; ε: growth efficiency

  Typical: $\mu = 1$ d^{-1}; $\varepsilon = 0.2$

$U \rightarrow$ B $\xrightarrow{G}$ B

$R \searrow CO_2$
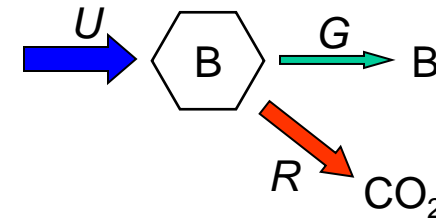
Ecosystem dynamics
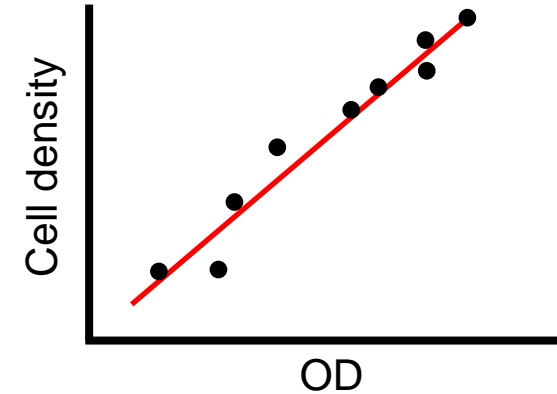
How is bacterial concentration measured?

Laboratory cultures

- Measure optical density and cell dry weight

Problems

- High cell densities required.
- Must be only cells (i.e., no debris or **detritus**)
- High predator abundance would also skew results.

⇒Technique does not work in the field!
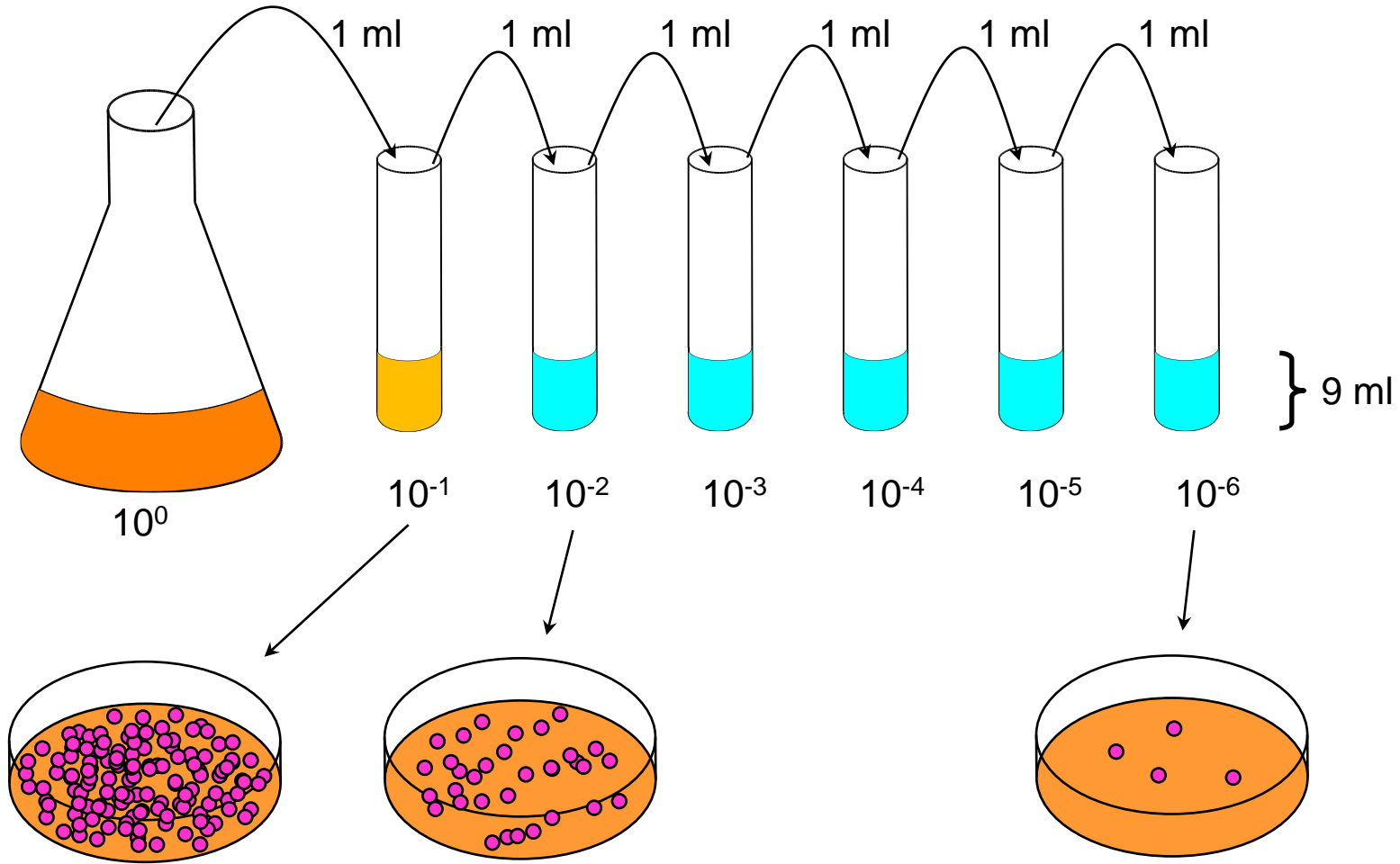
Dilution Plates

- Grow single cells on Petri plate until colonies are visible, then count colonies.
- Must use serial dilution so that colonies are in countable range.
- This method has a major problem. What is it?

Direct Counts

- Use microscope to directly count bacteria.

Problem: Bacteria in natural environments are very small and difficult to see and distinguish from detritus using standard light microscopy.

Dilution Plates

Statistically relevant colony density: 30 - 300

Technique largely used for isolation or water testing, such as coliform test.

Dilution Plate Calculations

N: Number of colonies on plate

V_S: Volume pipetted onto Petri plate.

D: Dilution factor for test tube plated out.

ρ: Concentration of cells in original sample (cells ml^{-1})
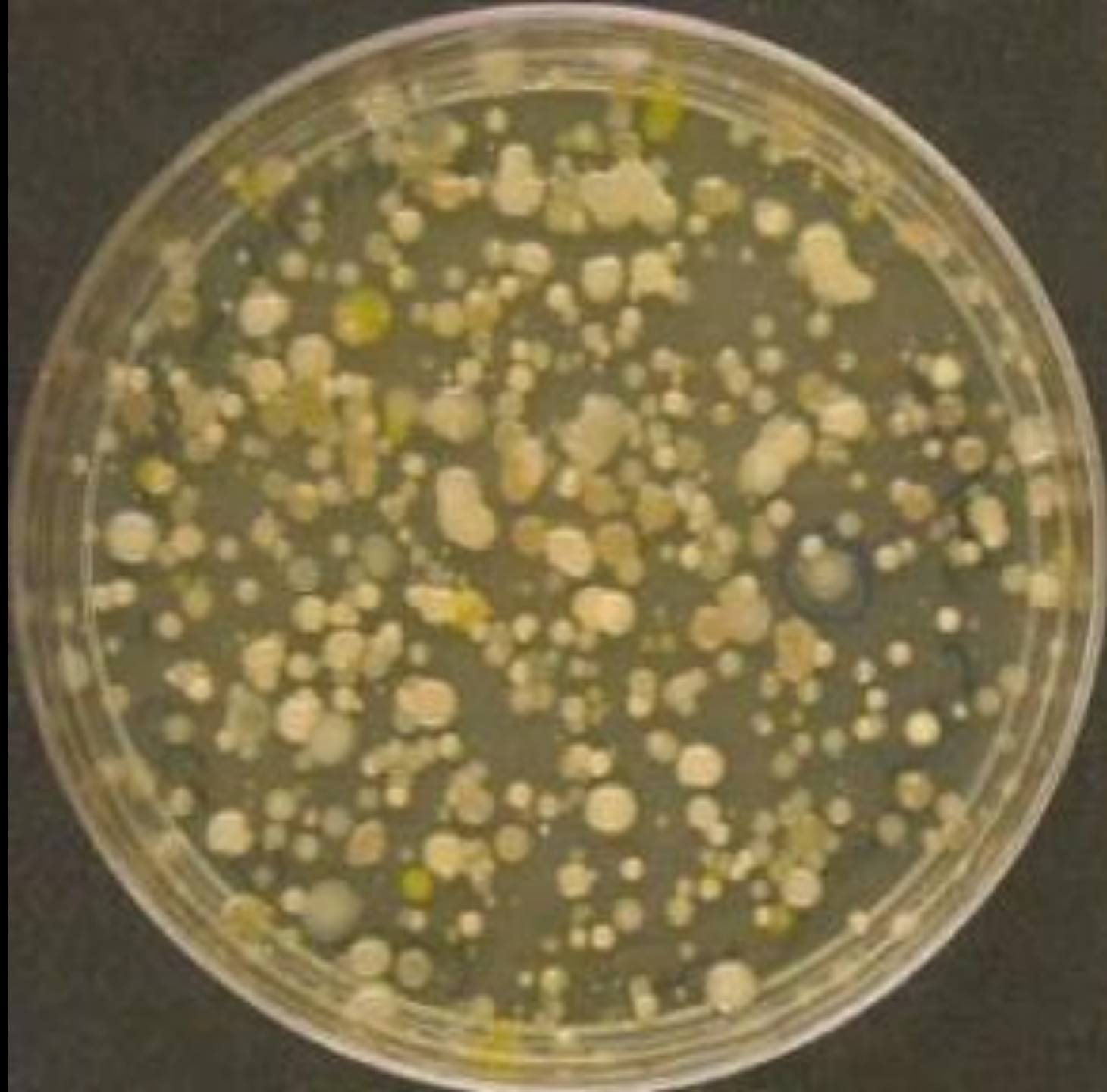
$$\rho = \frac{N}{V_S}\frac{1}{D}$$

Example:

N: 33

V_S: 100 μl

D: 10^{-4}

$$\rho = \frac{33}{0.1}\frac{1}{10^{-4}} = 3.3 \times 10^6 \, \text{cells ml}^{-1}$$

Fecal Coliform Counts

The abundance of fecal coliform bacteria are used as an *indicator* of fecal contamination of both drinking water and recreational water (i.e., swimming, shellfishing).

Fecal coliform bacteria inhabit the intestinal tracks of animals. While the indicator bacteria are typically not pathogens, they indicate that the water has become contaminated with fecal material, either by human or other animals.

Although it would be better to assay for pathogens directly (such as hepatitis), it is still too difficult to culture these organism quickly and reliably (or at all).

Basic method:
- Aseptically collect and filter water onto sterile filter.
- Place filter on sterile pad that contains medium for the culturing of fecal coliform bacteria (contains eosin-methylene blue dye)
- Incubate filter at 40ºC (or higher)
- Count colonies to determine colonies/100 ml water

EPA requirements (cfu/100ml):
- Drinking water: None
- Shell fishing: ≤ 14
- Swimming ≤ 200
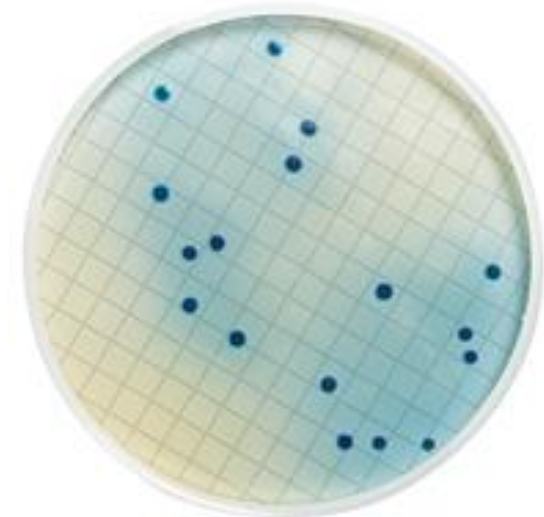
Some Drinking Water Pathogens

Viruses:
- Hepatitis
- Enterovirus
- Noroviruses

Bacteria:
- Cholera (*Vibrio cholera)*
- typhoid fever (*Salmonella typhi*)
- Fecal bacteria (often *Escherichia coli*)
- *Campylobacter*

Protists:
- Cryptosporidia
- Giardia

Falmouth Enterprise
7/18/07

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CLOSED AREA
NO SHELLFISHING

MARTHA V. SCANLON/ENTERPRISE
Woodneck Beach

Woodneck Creek Closed, Reopened Due To High Bacteria Counts

By MARTHA V. SCANLON

After closing last Wednesday due to high bacteria count, the creek at Woodneck Beach reopened Thursday for swimming.

Water samples from town beaches are tested by the Barnstable County Department of Health every week during the summer for bacteria levels. If a beach has a high bacteria count, it is closed for swimming, then retested daily until it passes.

Around 2:30 Wednesday, not long after the beach closing was posted for high bacteria counts, many people were still lying on the beach and some were wading in the creek's waters. The ocean side of Woodneck Beach remained open.

The closing was posted on a yellow sign at the entrance to the creek beach.

Jessica A. Poppe of Green Pond Road, East Falmouth, a parking attendant at Woodneck, said that she saw many people drive into the beach parking lot, but leave once they read the sign.

"It's definitely not as busy as usual, especially considering it's so hot out," she said.

After it rains, bacteria from nearby roads and houses can wash into the water, causing the bacteria count to be high. Bacteria is usually flushed away during the day by the tide.

There have been two other brief beach closings so far this summer.

According to the Barnstable County beach water quality report, Old Silver Beach failed on June 20 and was reopened the next day and Woodneck Beach failed on June 13 and was also reopened the next day.

Test results for Falmouth Beaches are available at barnstablecountyhealth.org/bsfalmouth.htm.

Direct Bacterial Counts

Challenges with Direct Count Method

- Natural samples contain low concentrations of bacteria (10^6 cells ml^{-1})

    ⇒ **Must concentrate bacteria**

- Bacteria are small (0.2 - 1 μm) so difficult to see and differentiate from detritus using microscope with normal or phase contrast lighting techniques.

   ⇒ Must stain with fluorescent dye and use epifluorescence microscopy.

Procedure outline

- Incubate water sample with fluorescent dye.

- Concentrate sample onto **Black** 0.2 μm filter.

- Place filter on slide, and count bacteria in grid

- Calculate bacterial numbers.
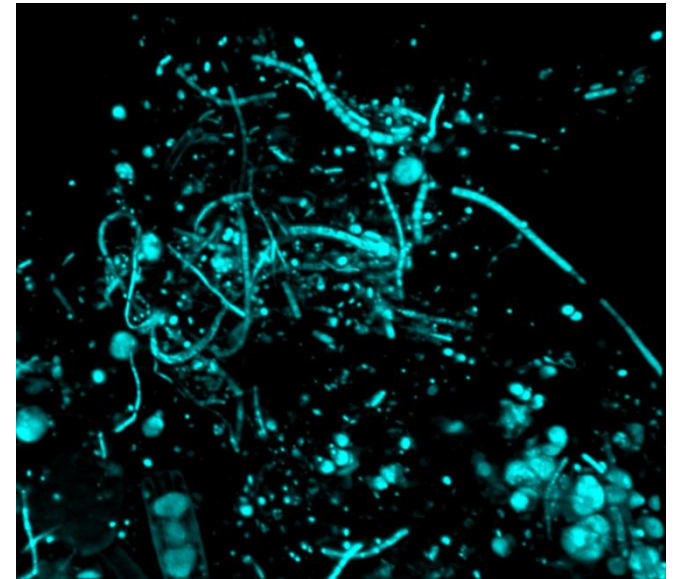
Epifluorescence Microscopy

Fluorescence

- Compound is "excited" at a particular wavelength of light (usually in the UV)

- Compound then emits light at a different, lower, wavelength.
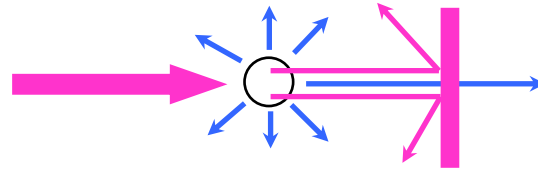
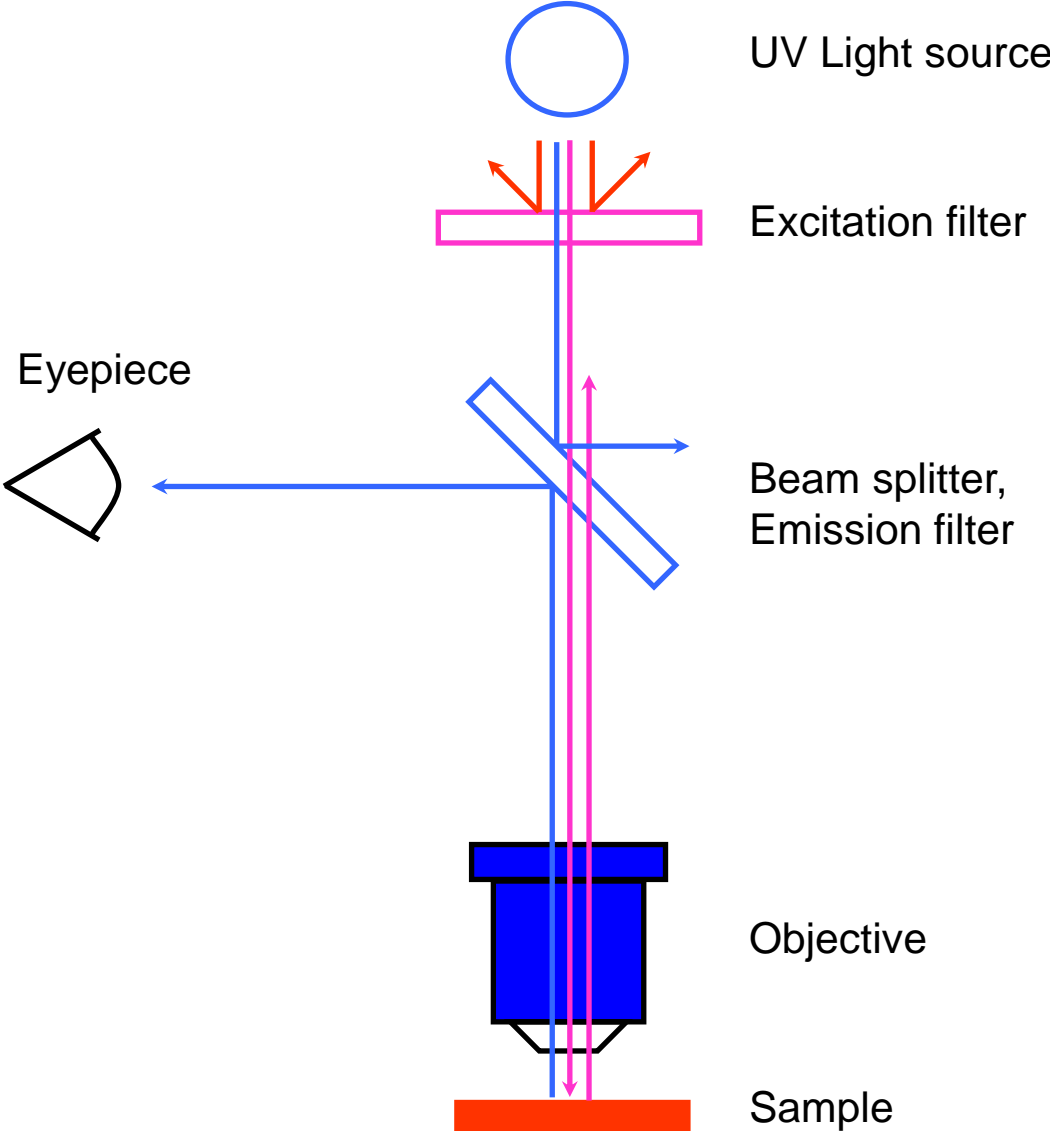- Advantage: contrast is extremely high, which allows detection of weak light.

Dyes used

- Acridine orange (AO)

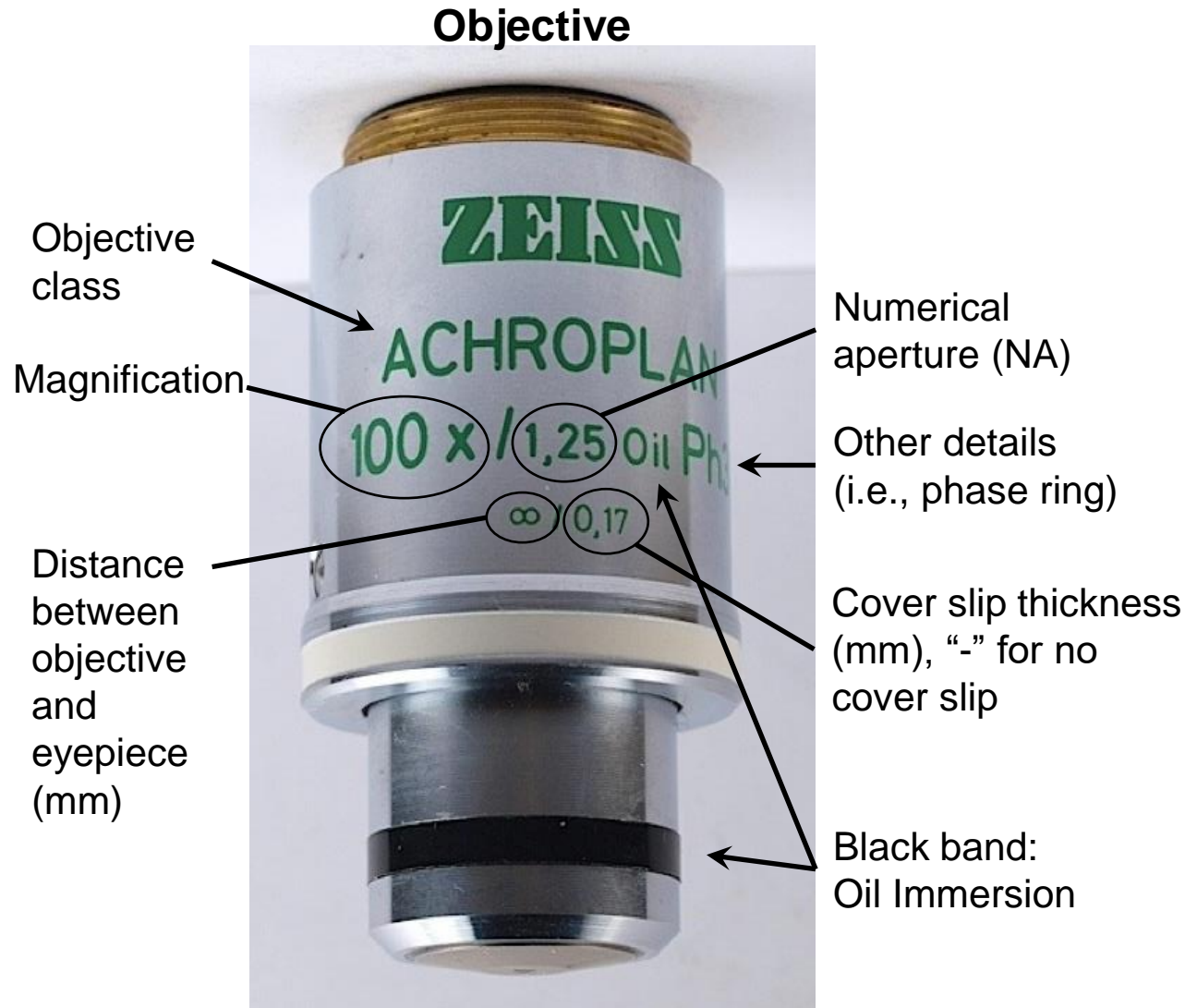- DAPI (4'6-diamidino-2-phenylindole)

Mechanisms

- AO fluoresces when bound to DNA or RNA. Cells appear orange.

- DAPI fluoresces when bound to DNA and is more specific. Cells appear blue.

Epifluorescence Details

UV Light source

Excitation filter

Eyepiece

Beam splitter,
Emission filter

Objective

Sample

Objective & Eyepiece Descriptors

Objective

Objective class

Magnification

Distance between objective and eyepiece (mm)

Numerical aperture (NA)

Other details (i.e., phase ring)

Cover slip thickness (mm), "-" for no cover slip

Black band: Oil Immersion

Eyepiece

Magnification

Field of view

Total magnification: Eyepiece $\times$ Objective

Maximum magnification: $1000 \times$ NA

Minimum magnification: $500 \times$ NA

Slide Preparation for DAPI
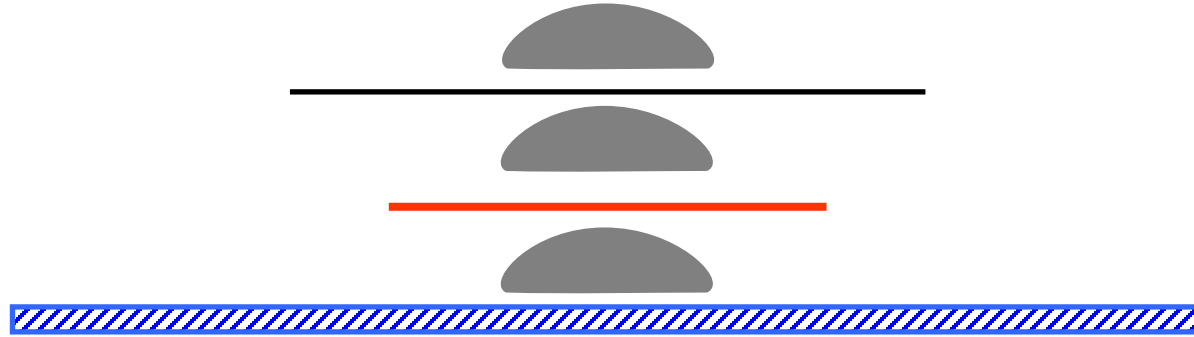
Drop of immersion oil
Cover slip
Drop of immersion oil
Filter, bacteria side up!
Drop of immersion oil
Microscope Slide

Notes:

- Place filter so that bacteria are facing up.

- Use small drops of immersion oil.

- Cover slips stick together. If you have more than one, you will not be able to focus well.

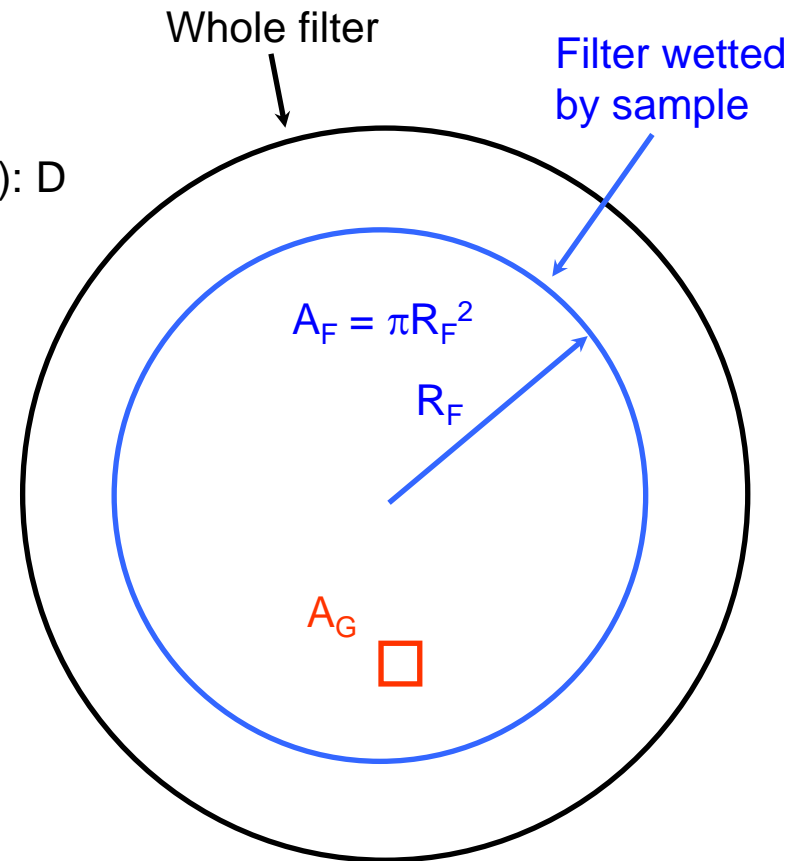- Label slide.

Cell Density Calculations

Known or measured

- Volume of sample filtered: V_S

- Dilution Factor (from adding preservative): D

- Area of filter occupied by sample: A_F

- Area of grid in field of view: A_G

- Average number of cells grid^{-1}: N

Cell Concentration

- Cell Conc: ρ

$$\rho = \frac{\dfrac{A_F}{A_G} N}{V_S D}$$

Whole filter

Filter wetted by sample

$$A_F = \pi R_F^2$$

R_F

A_G

What is the main assumption in this calculation?