Basic Water Microbiology and Microscopy

Tom O'Connor, PE

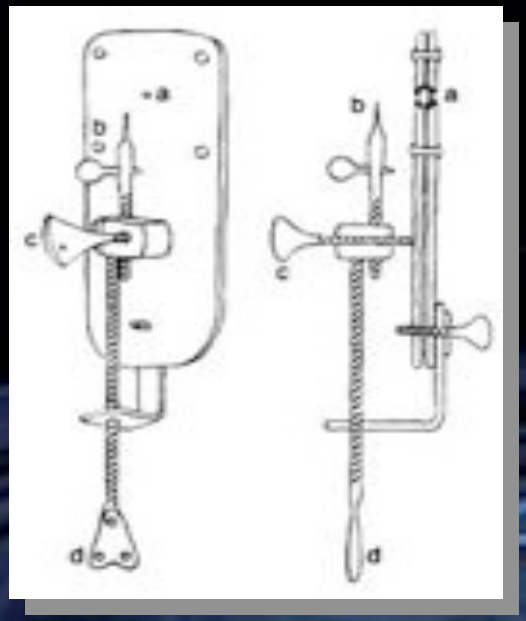
Discovery of the Microbial World

"I have had several gentlewomen in my house, who were keen on seeing the little eels in vinegar; but some of them were so disgusted at the spectacle, that they vowed never to use vinegar again.

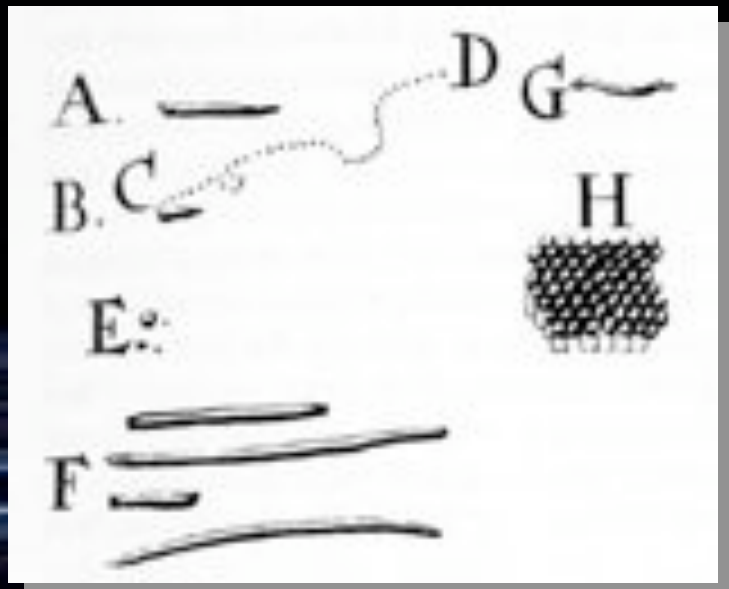
But what if one should tell such people in future that there are more animals living in the scum on the teeth in a man's mouth, than there are men in a whole kingdom?"

Antonj van Leeuwenhoek
1632-1723

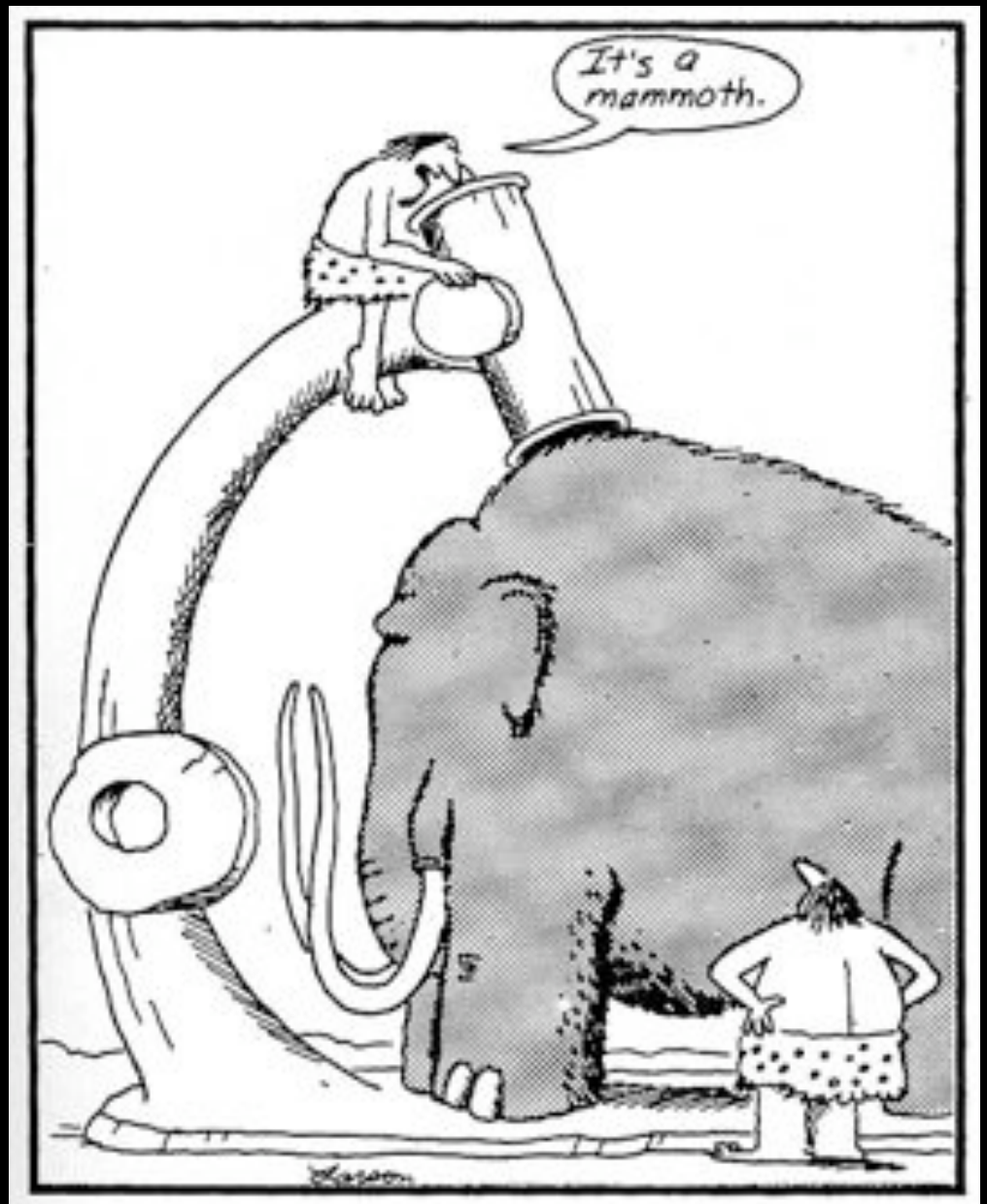
Leeuwenhoek's Microscope



50 to 300X magnification

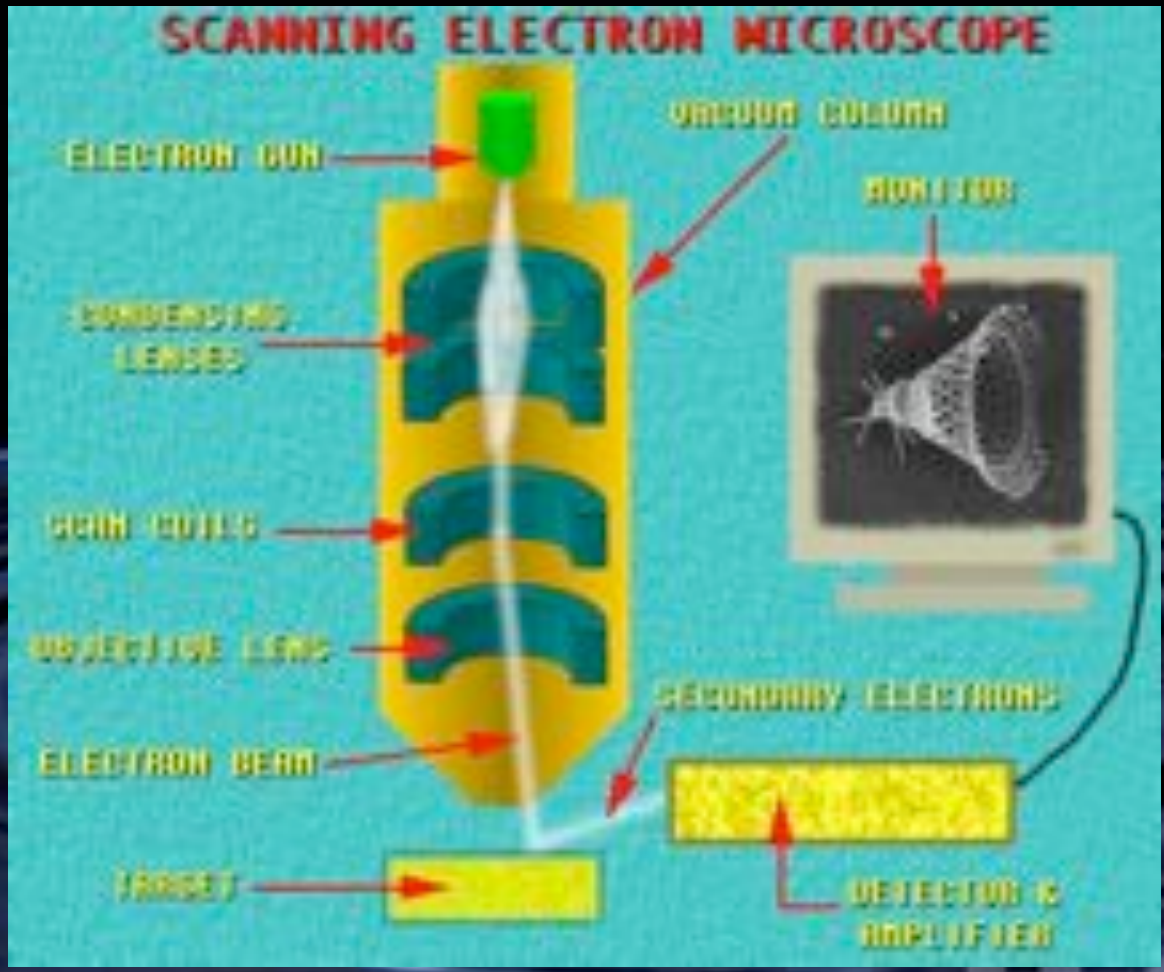


"animalcules," or little animals

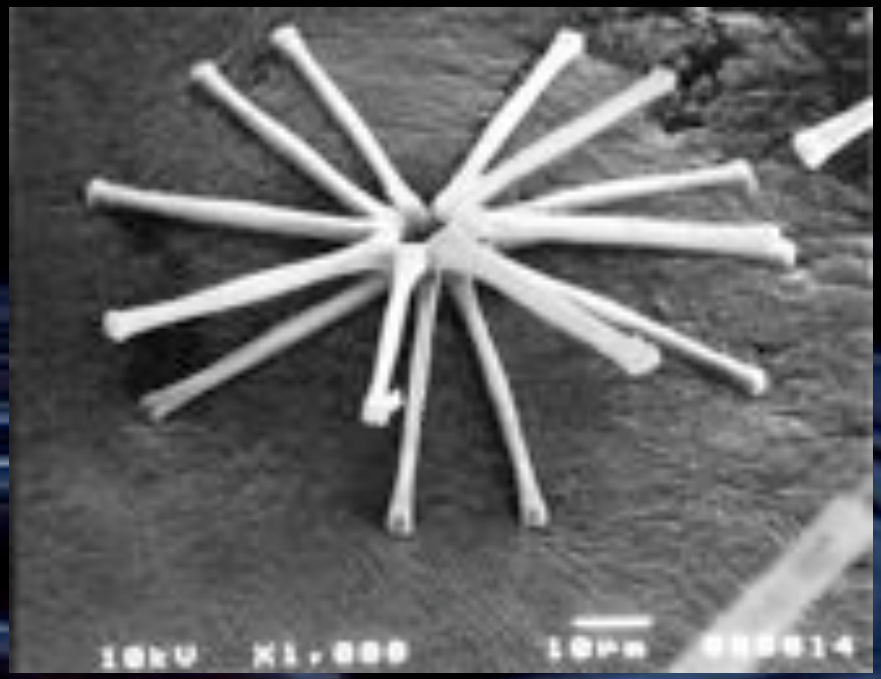


Early microscope

Scanning Electron Microscpe



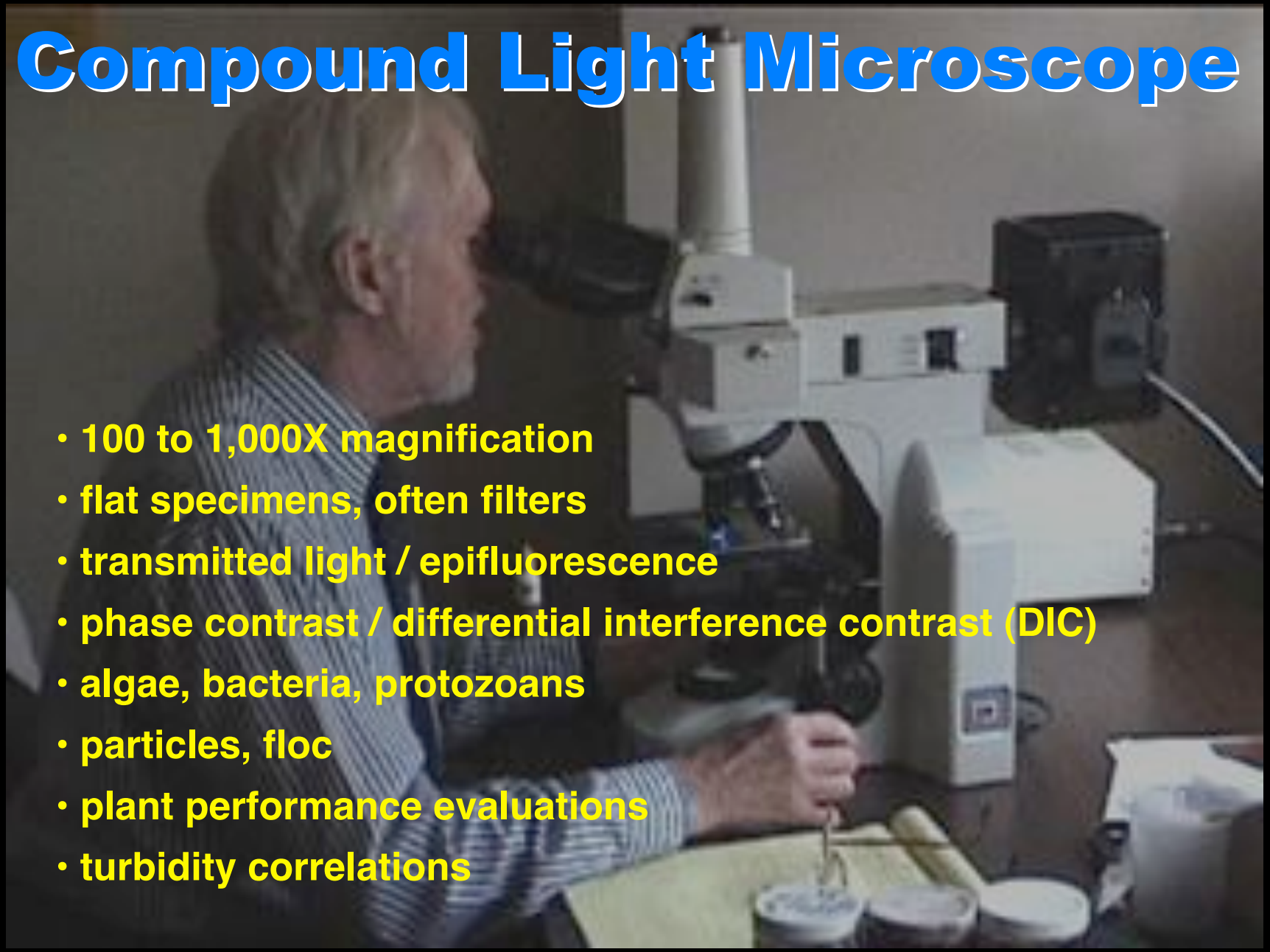
Scanning Electron Microscpe




ELECTRON
MICROSCOPE
LAB

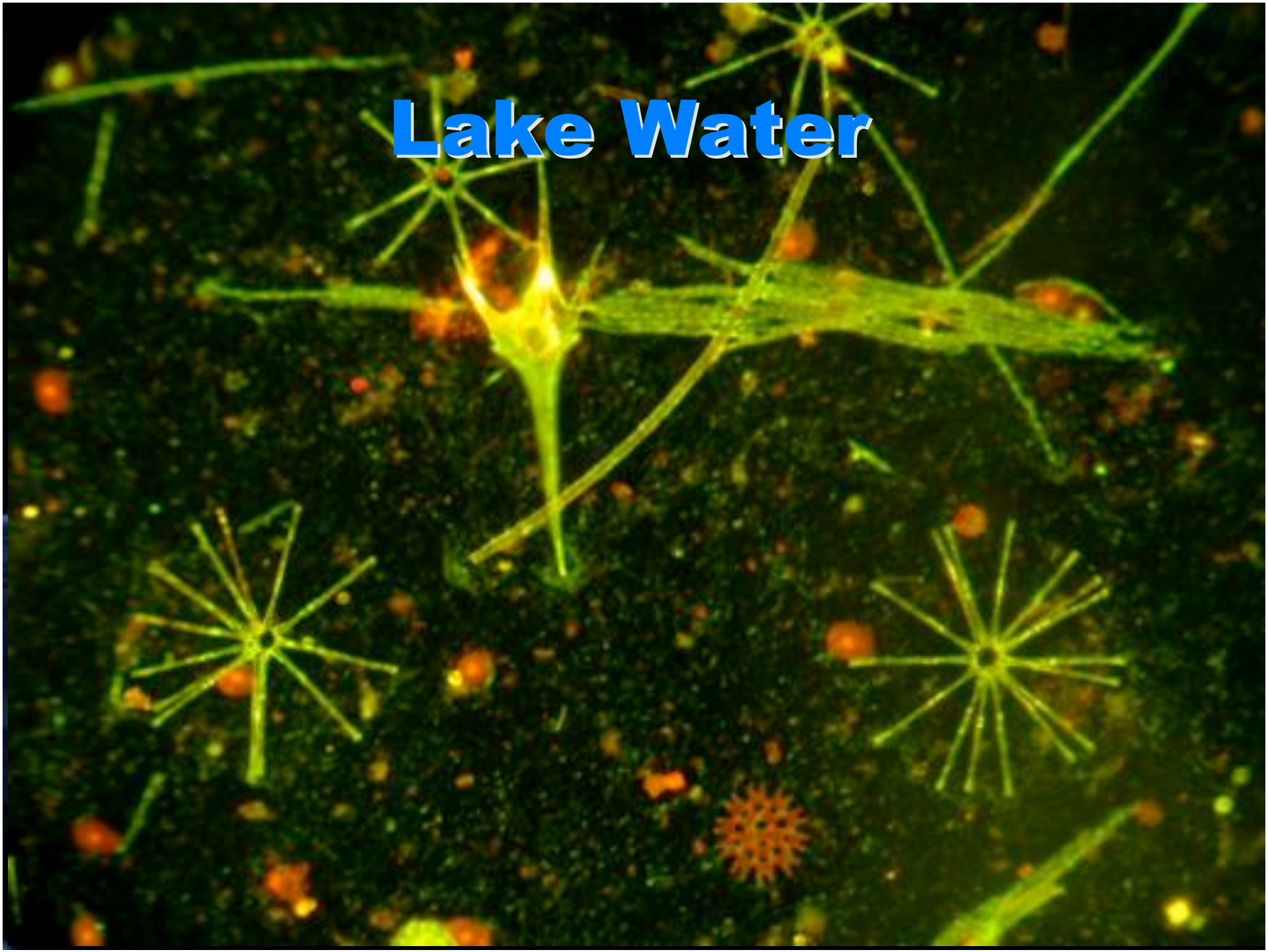
Compound Light Microscope

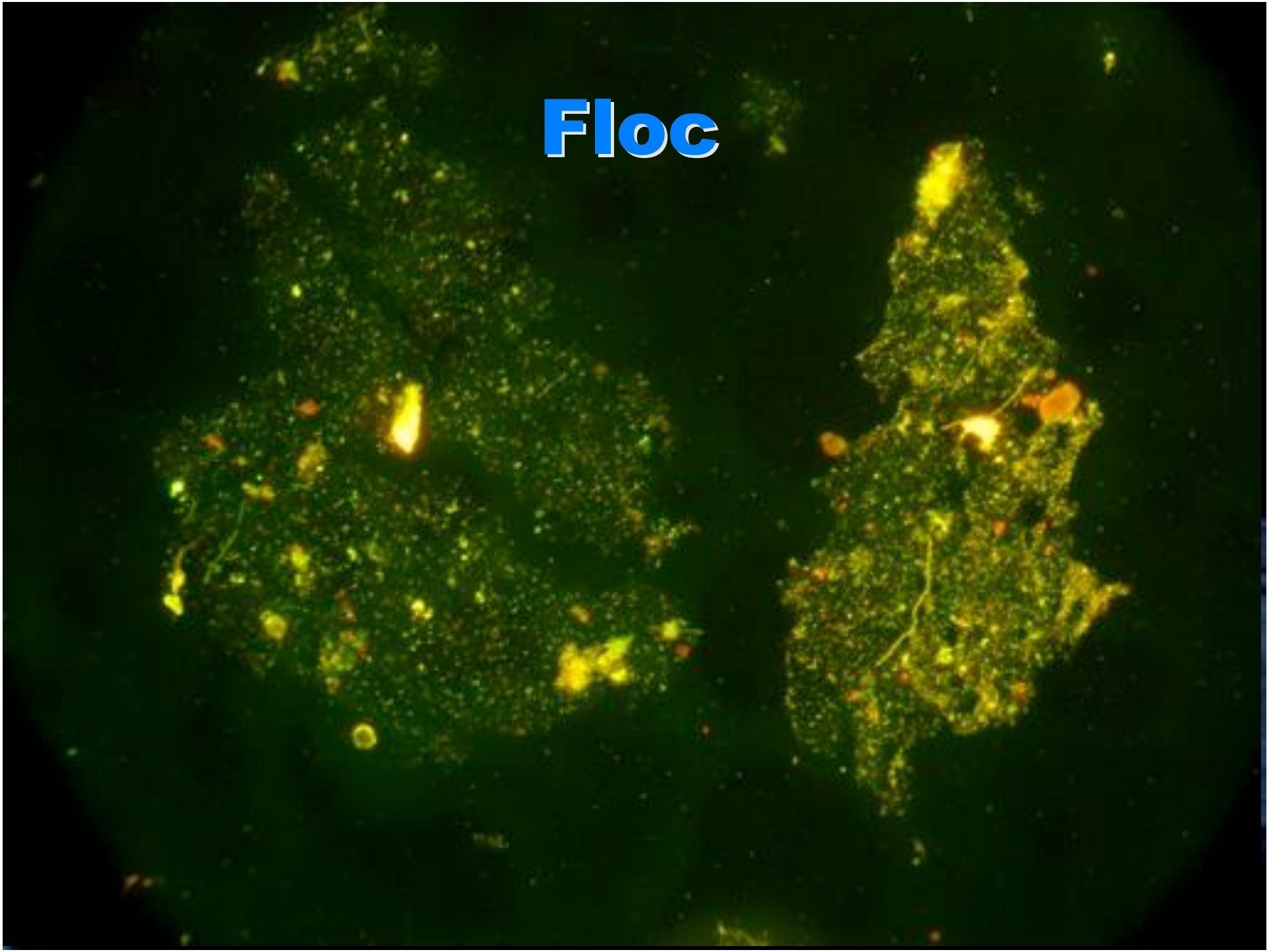
Compound Light Microscope



- 100 to 1,000X magnification
- flat specimens, often filters
- transmitted light / epifluorescence
- phase contrast / differential interference contrast (DIC)
- algae, bacteria, protozoans
- particles, floc
- plant performance evaluations
- turbidity correlations

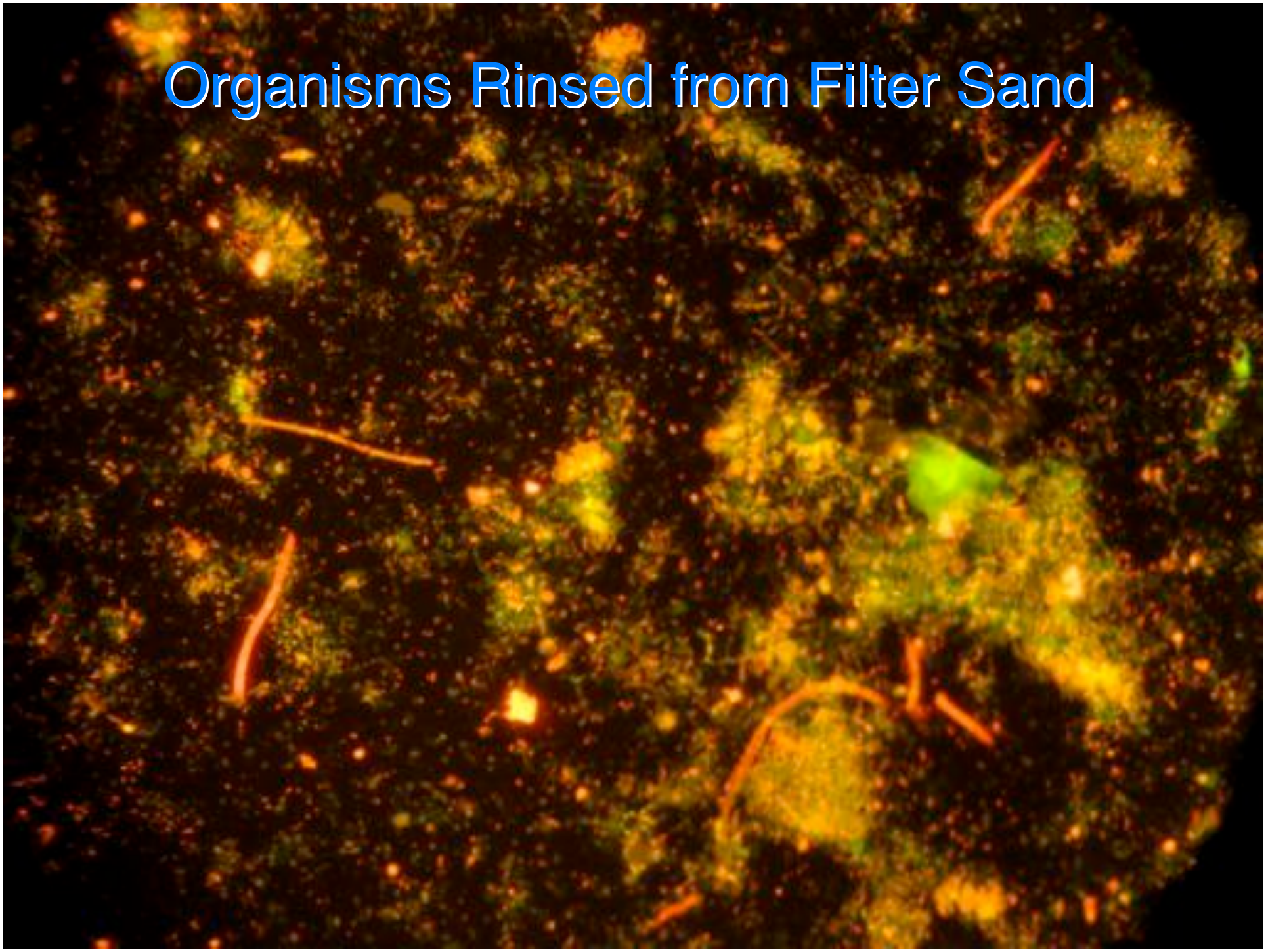
Transmitted Light to Epifluorescence

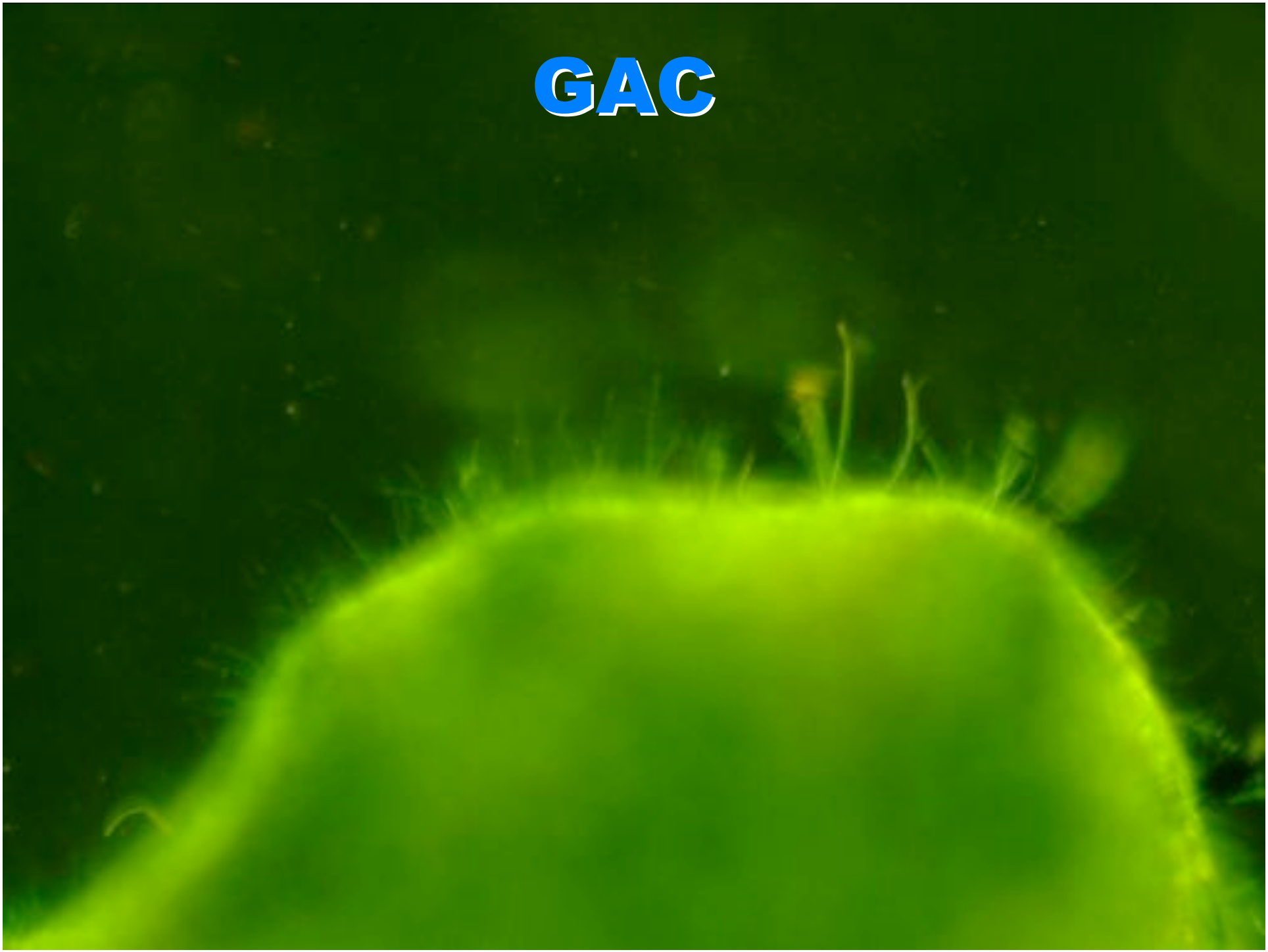

Lake Water

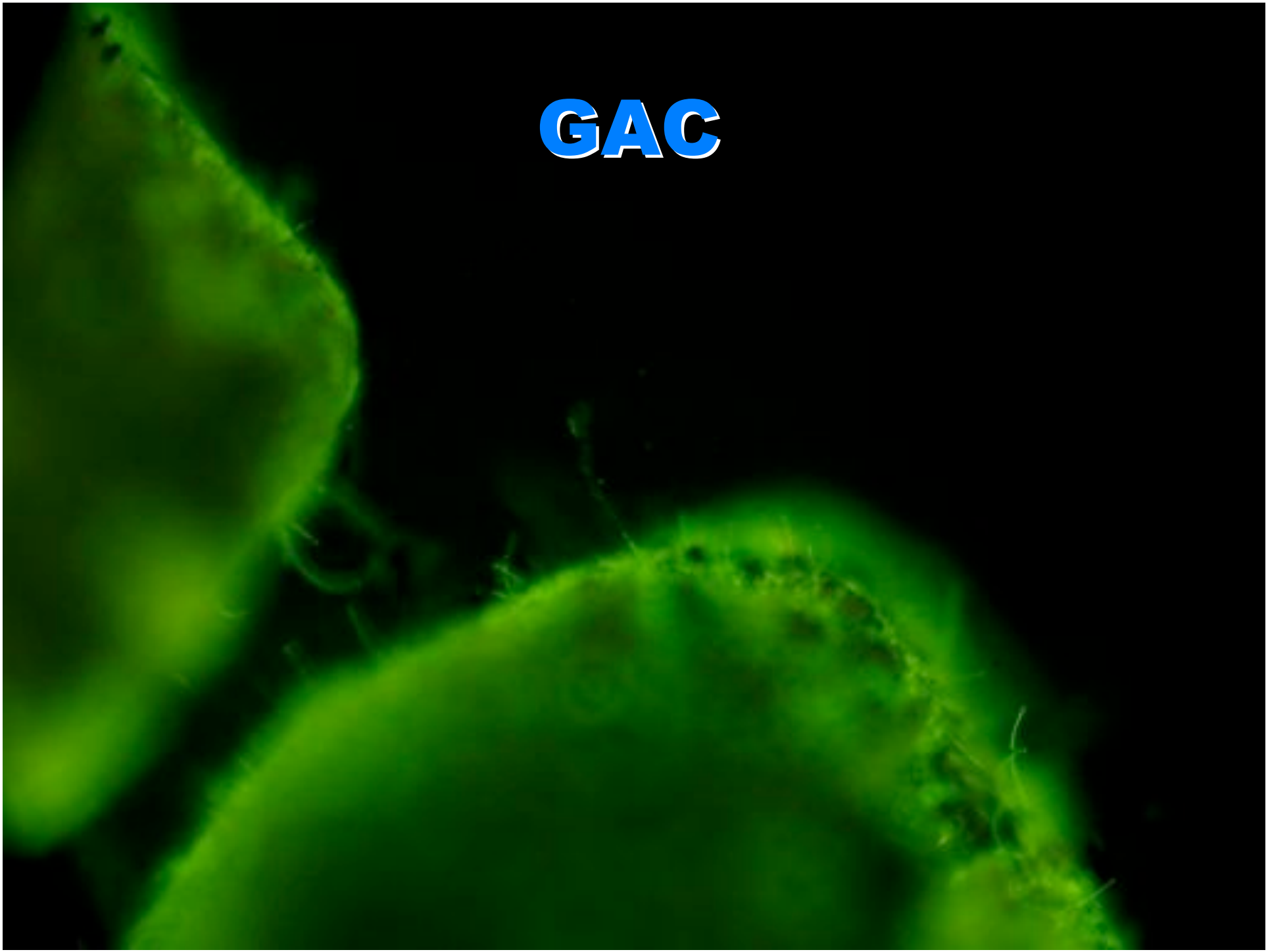

Floc


Filtered Water

Organisms Rinsed from Filter Sand




GAC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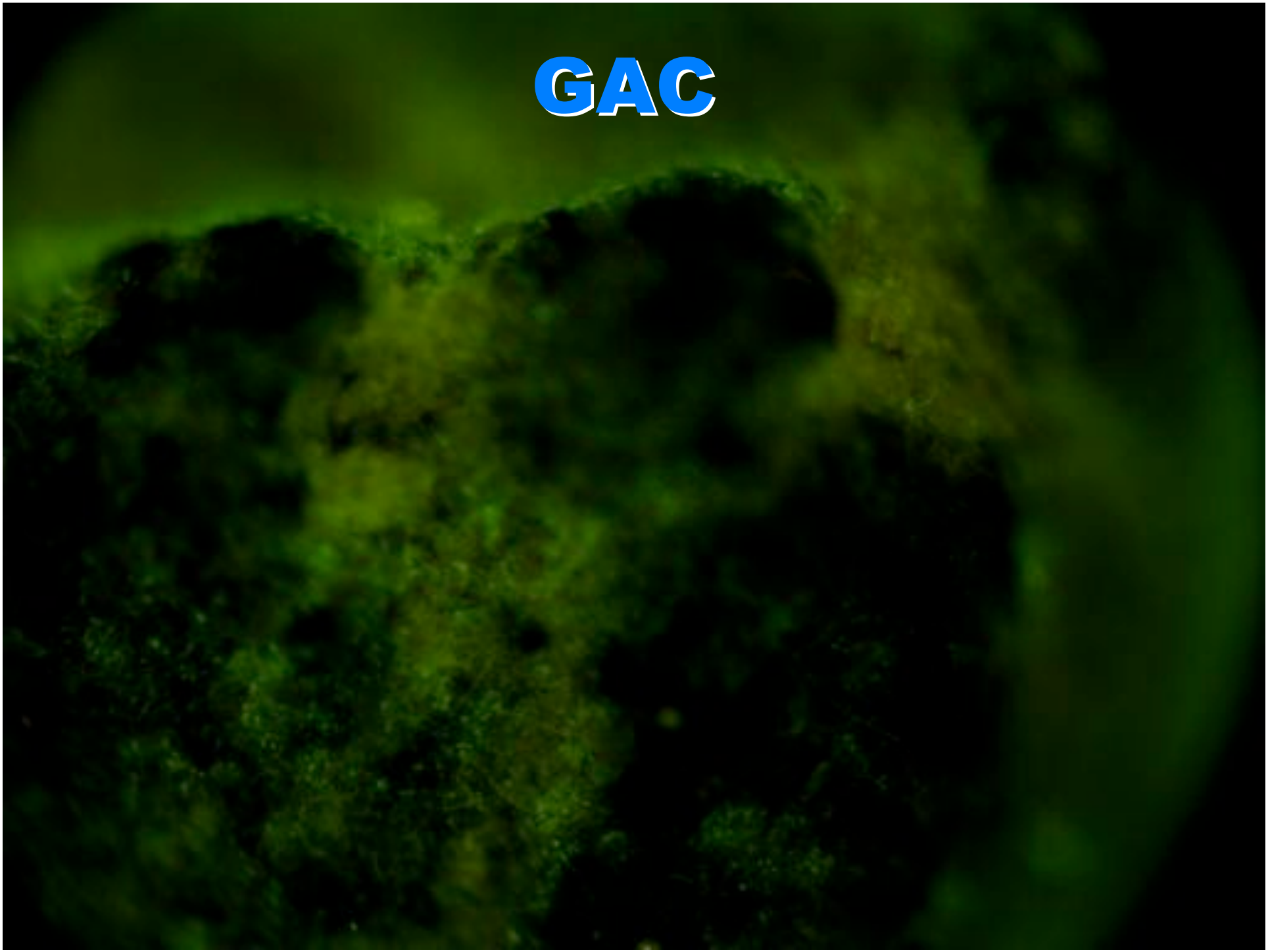
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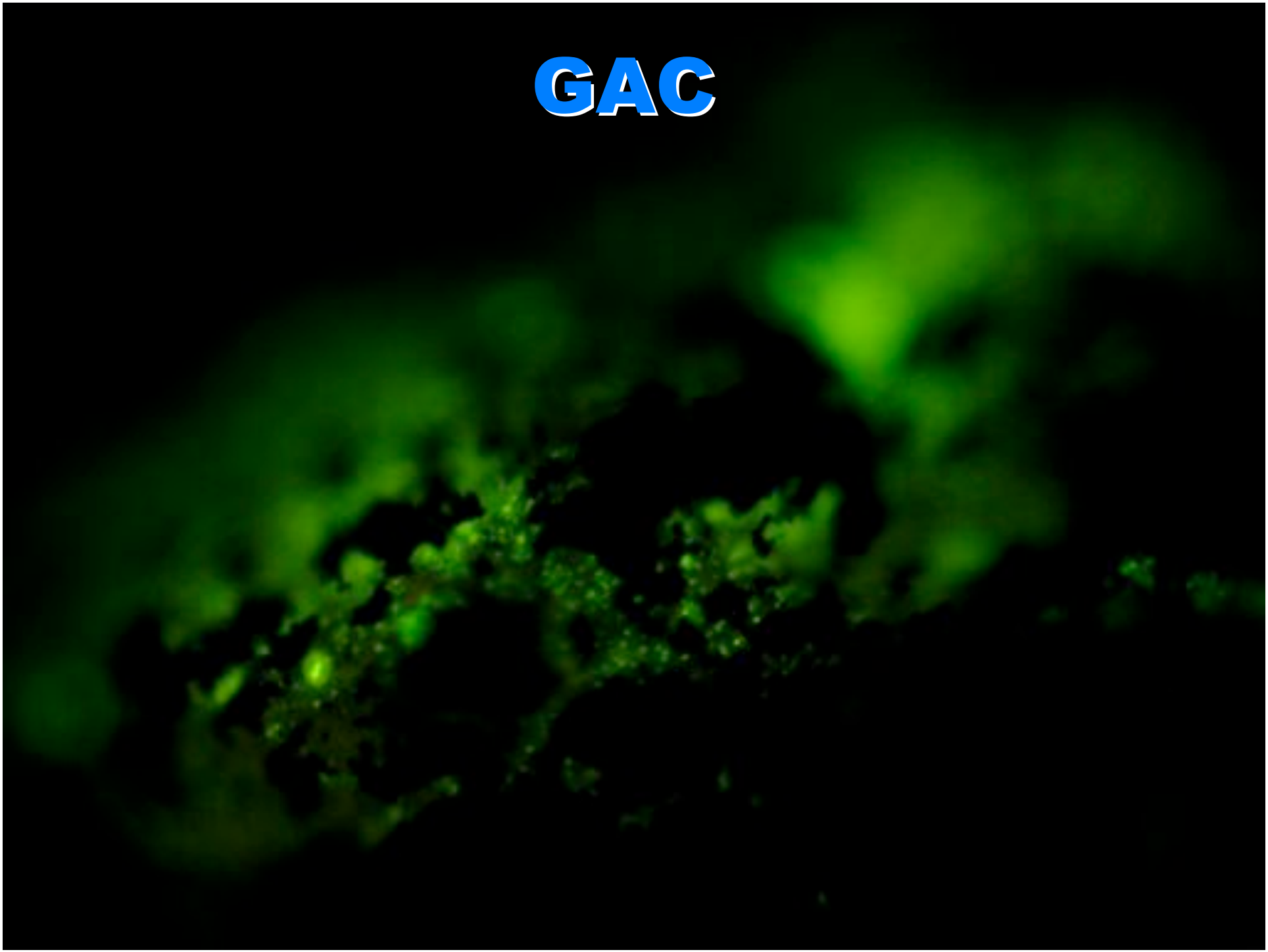
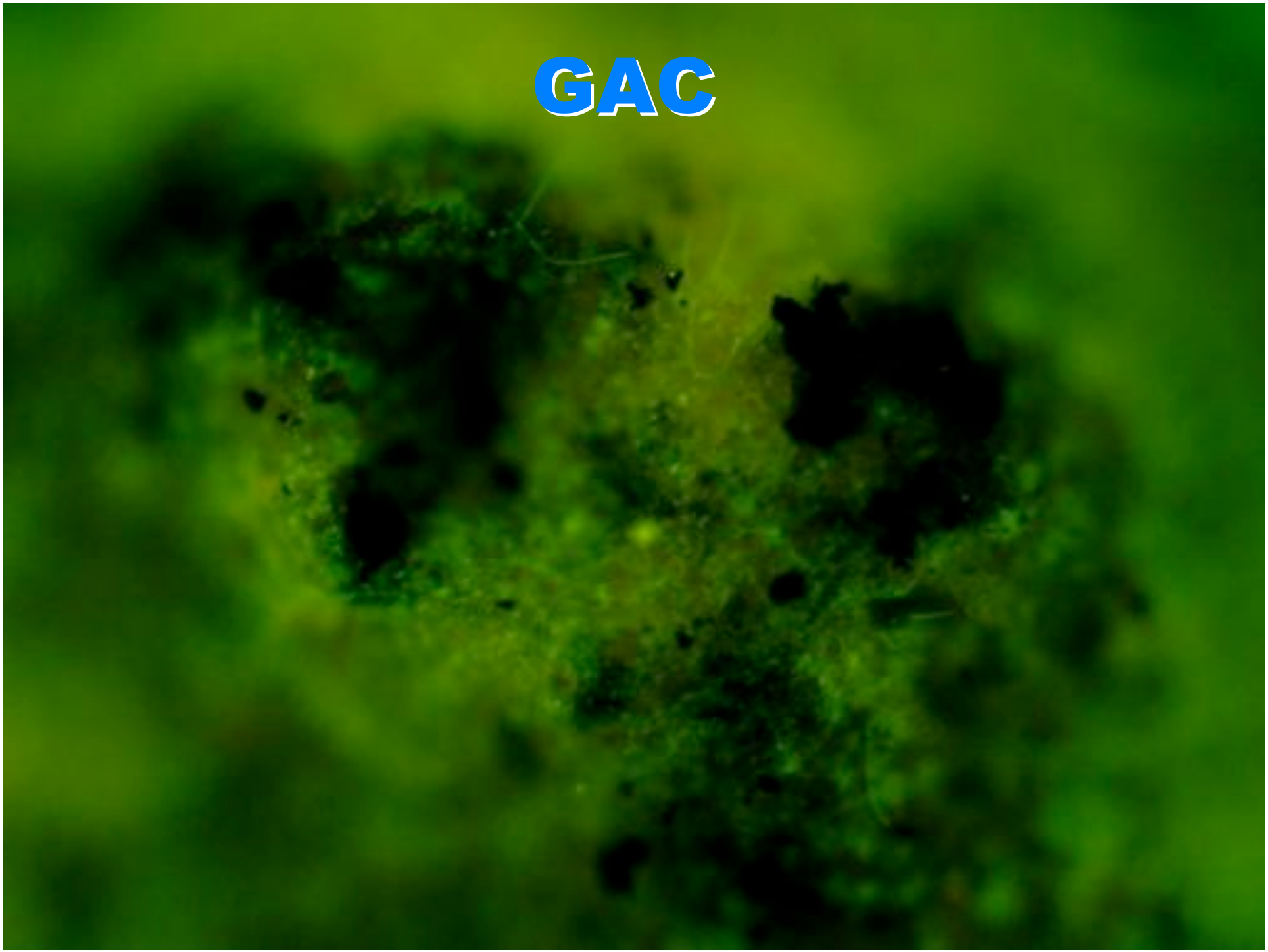

GAC


GAC


GAC

Stereo and Computer Scopes

Stereo and Computer Scopes

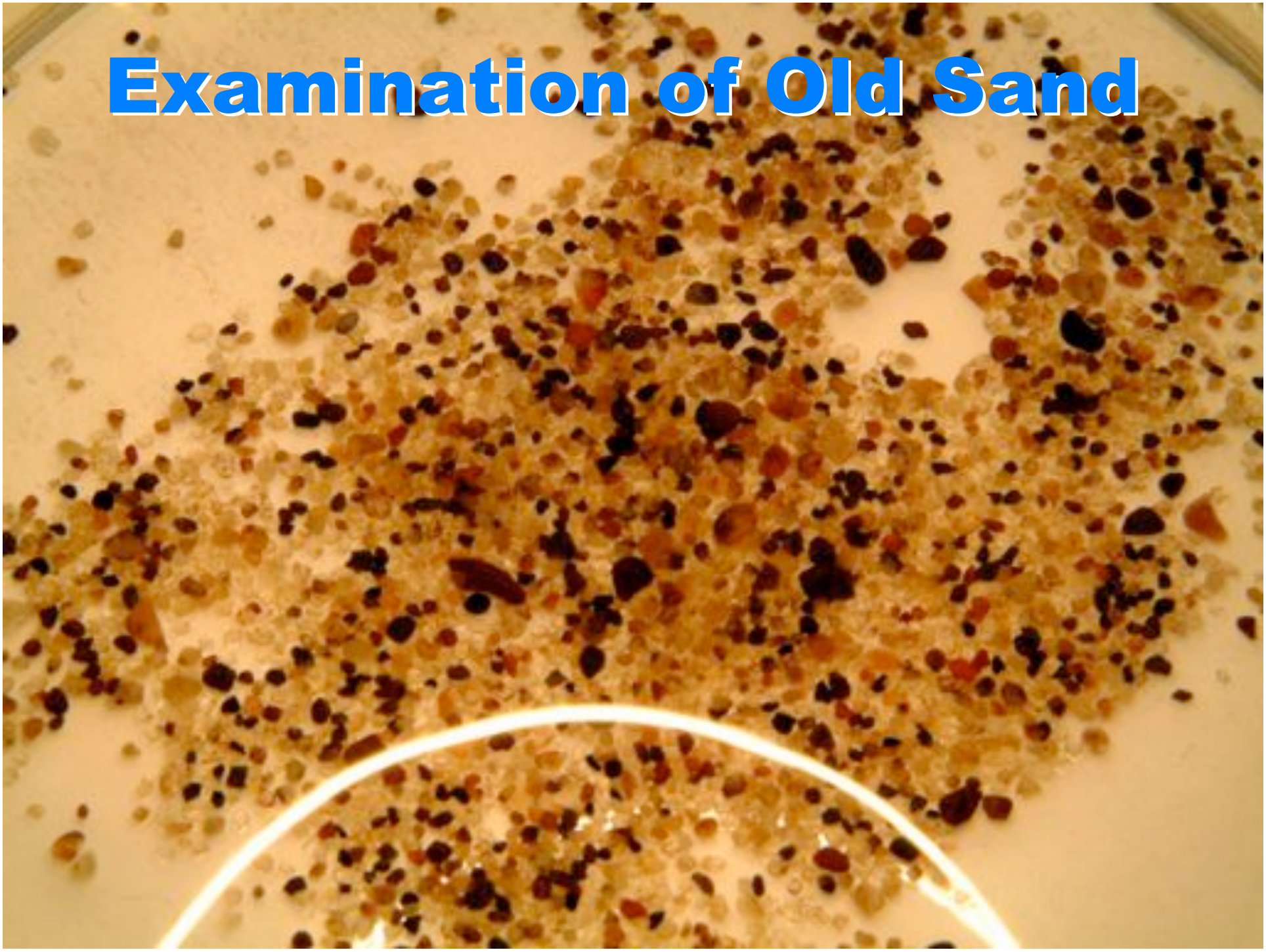
- three-dimensional specimens
- top- and bottom-illumination
- protozoans, macroinvertebrates
- media from filters, ion exchange
- GAC, PAC
- pipes

Examination of Old Sand

Examination of Old Sand

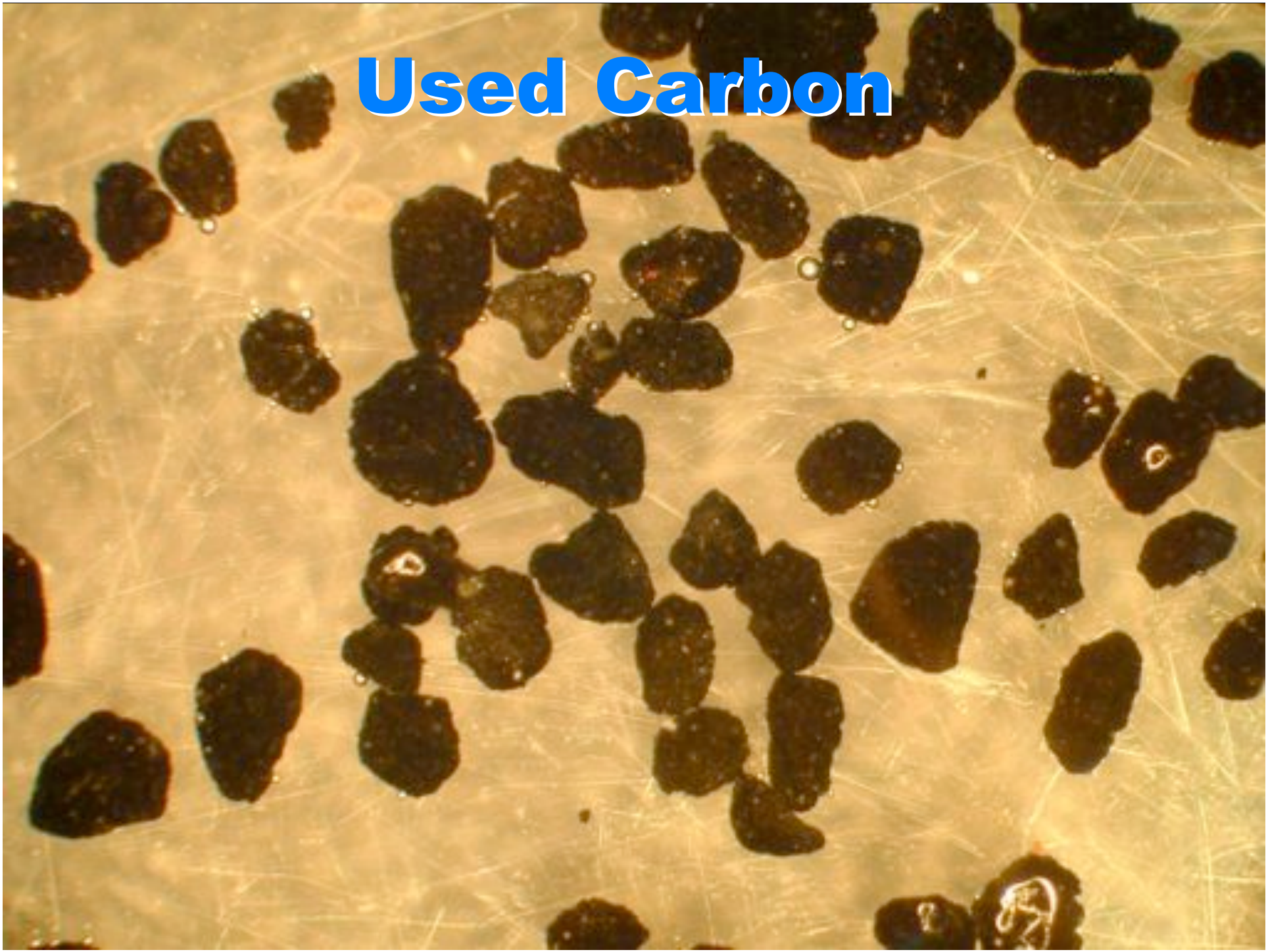
Examination of Old Sand

Examination of Old Sand

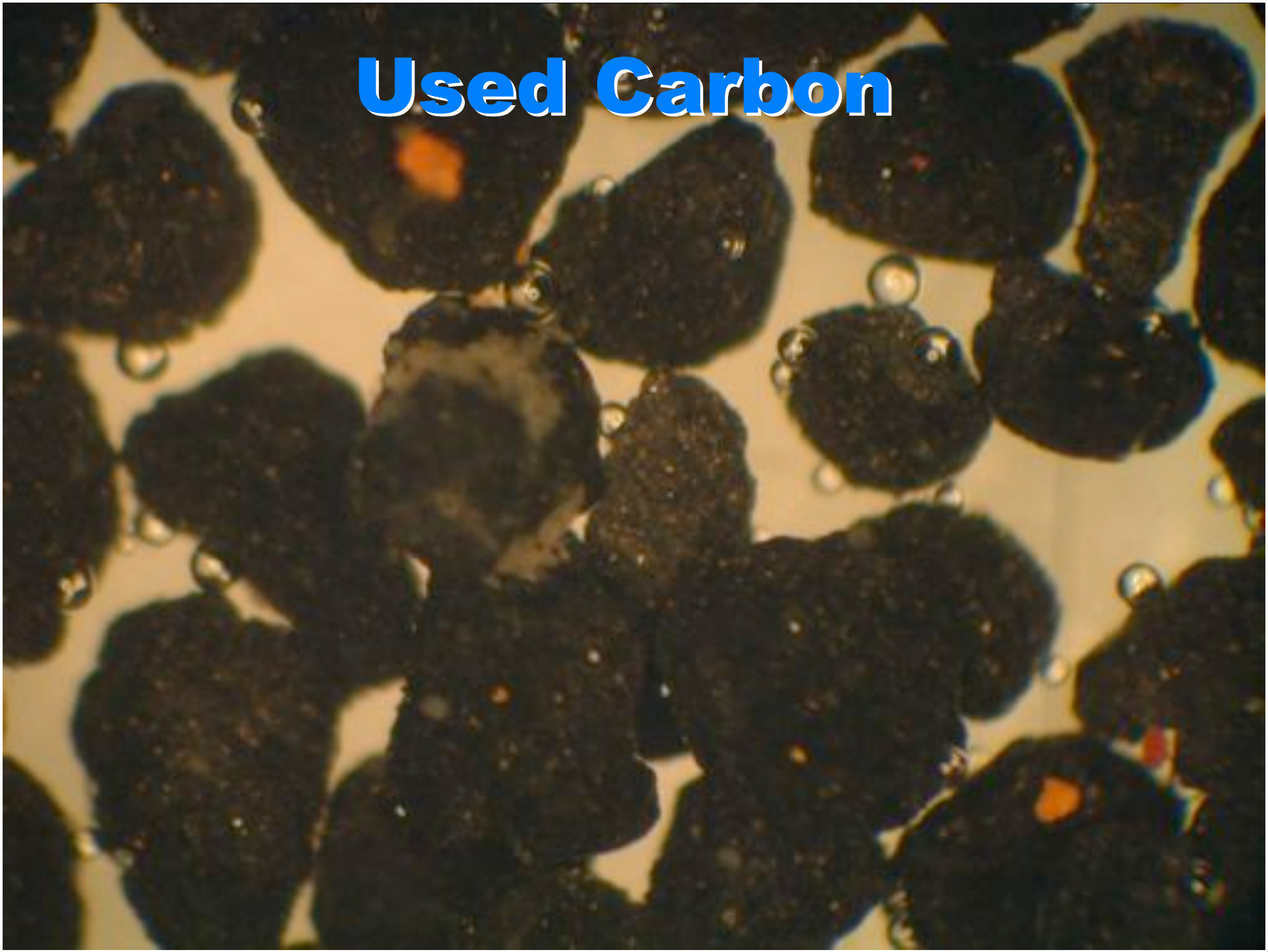


Examination of Old Sand

Used Carbon



Used Carbon

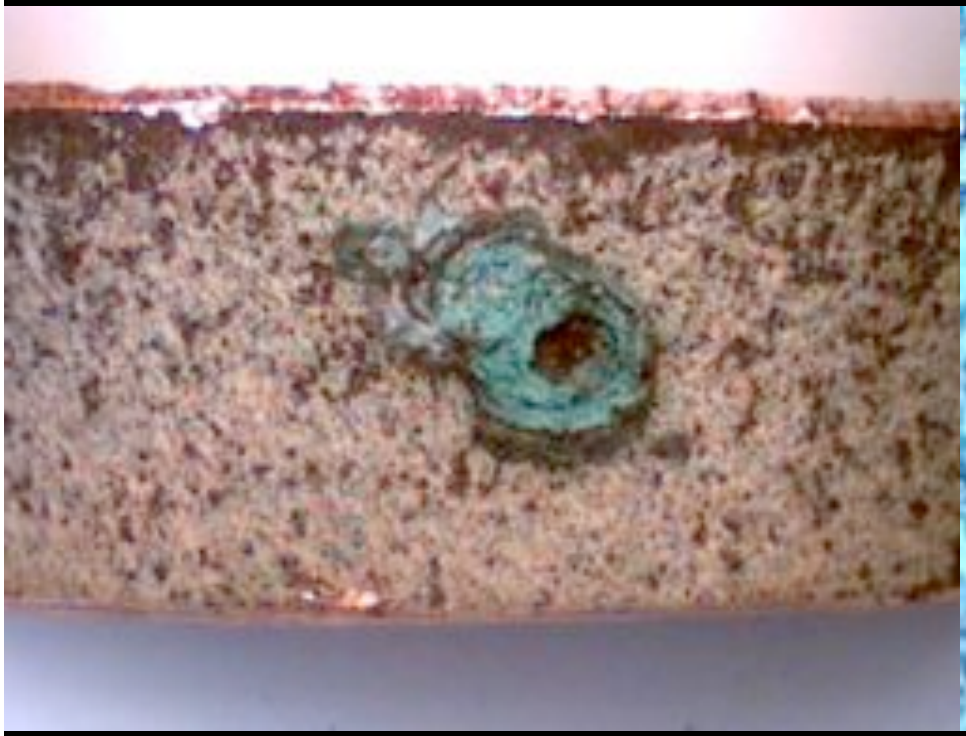


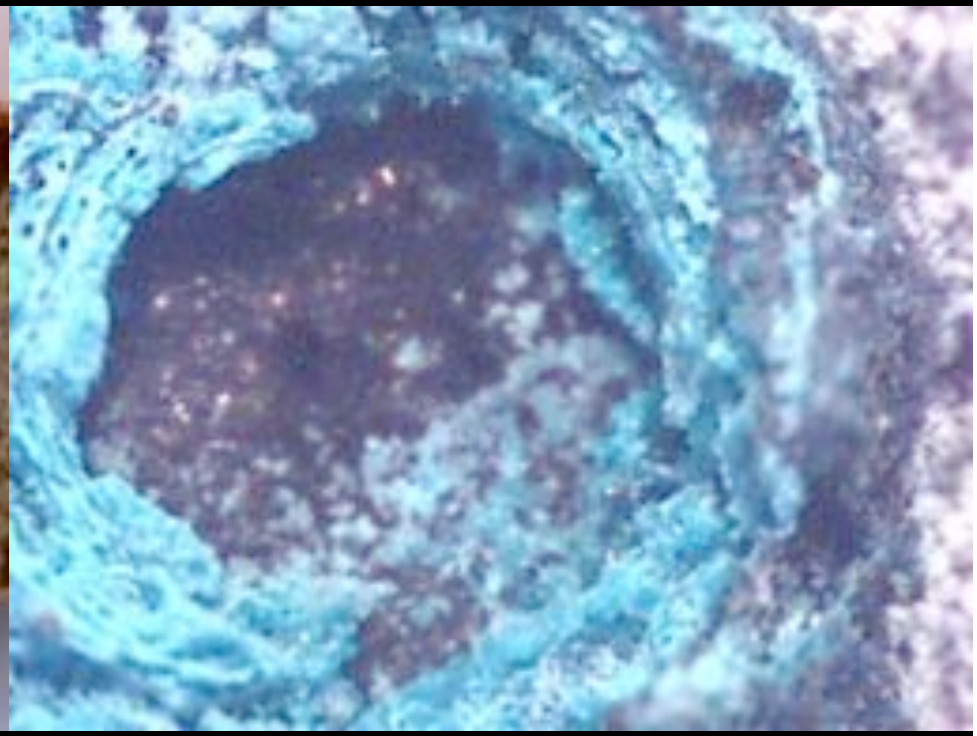
Stereo Scope

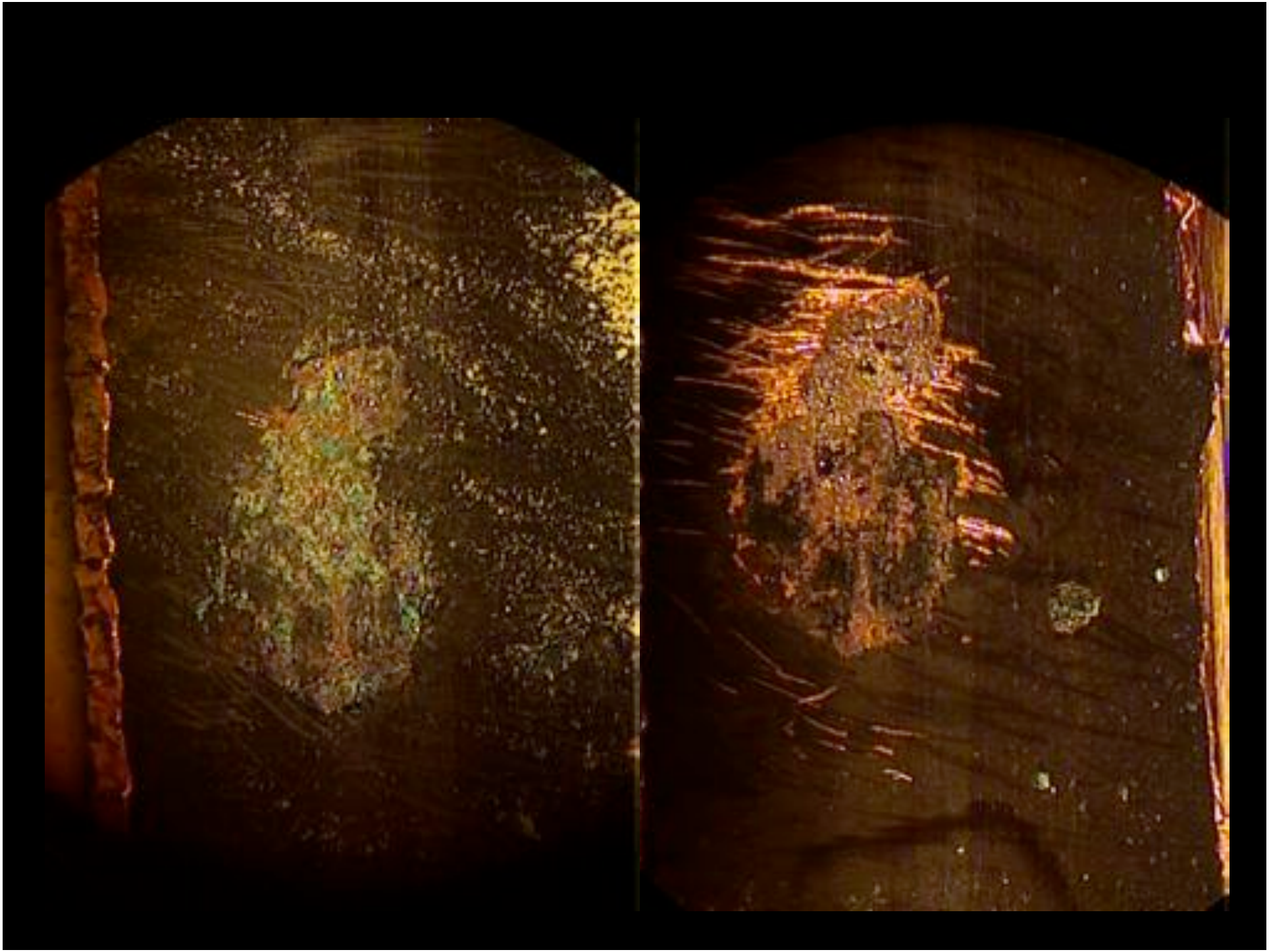
Pitting Corrosion

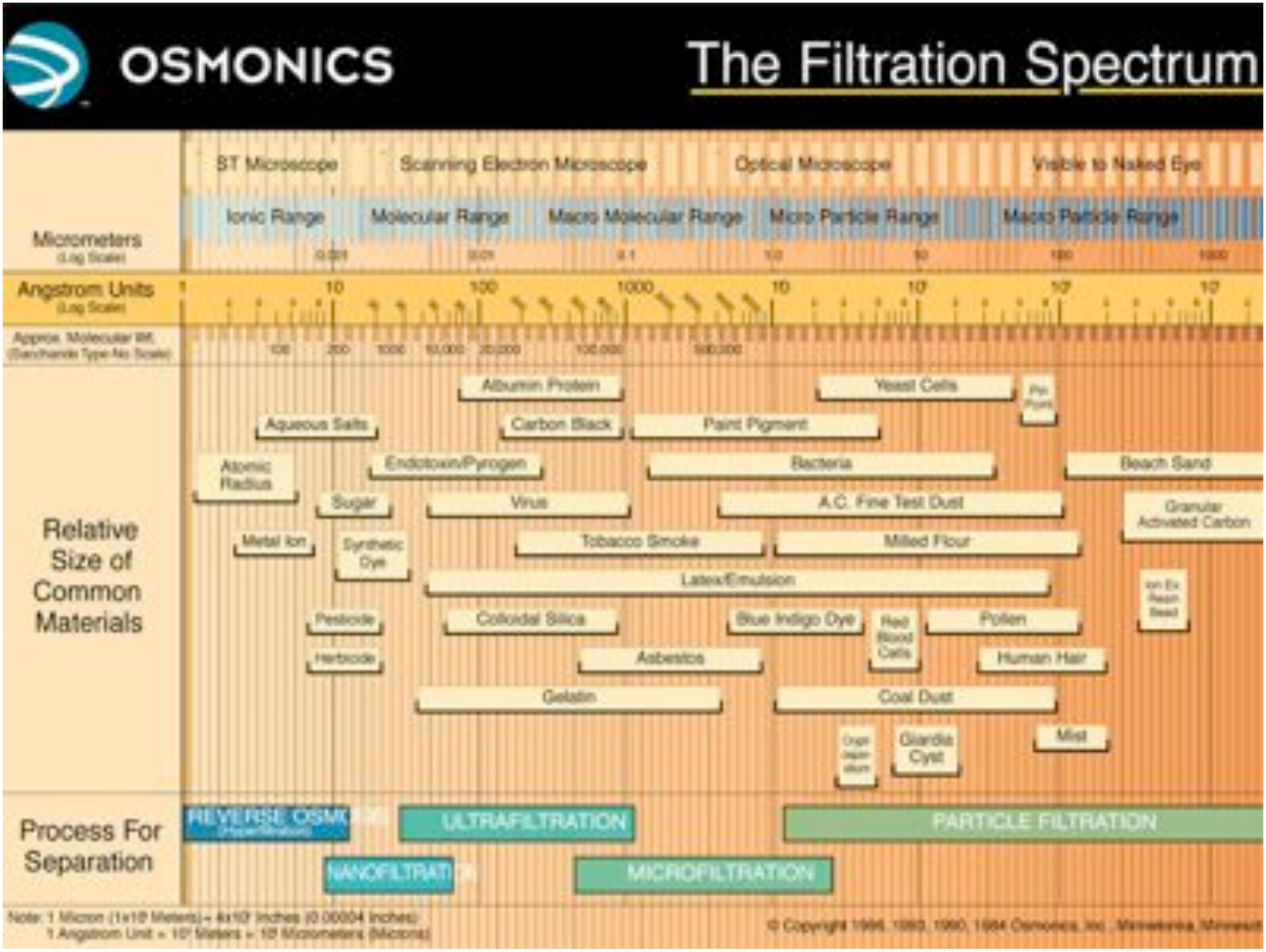

The Pit

Pit Pics

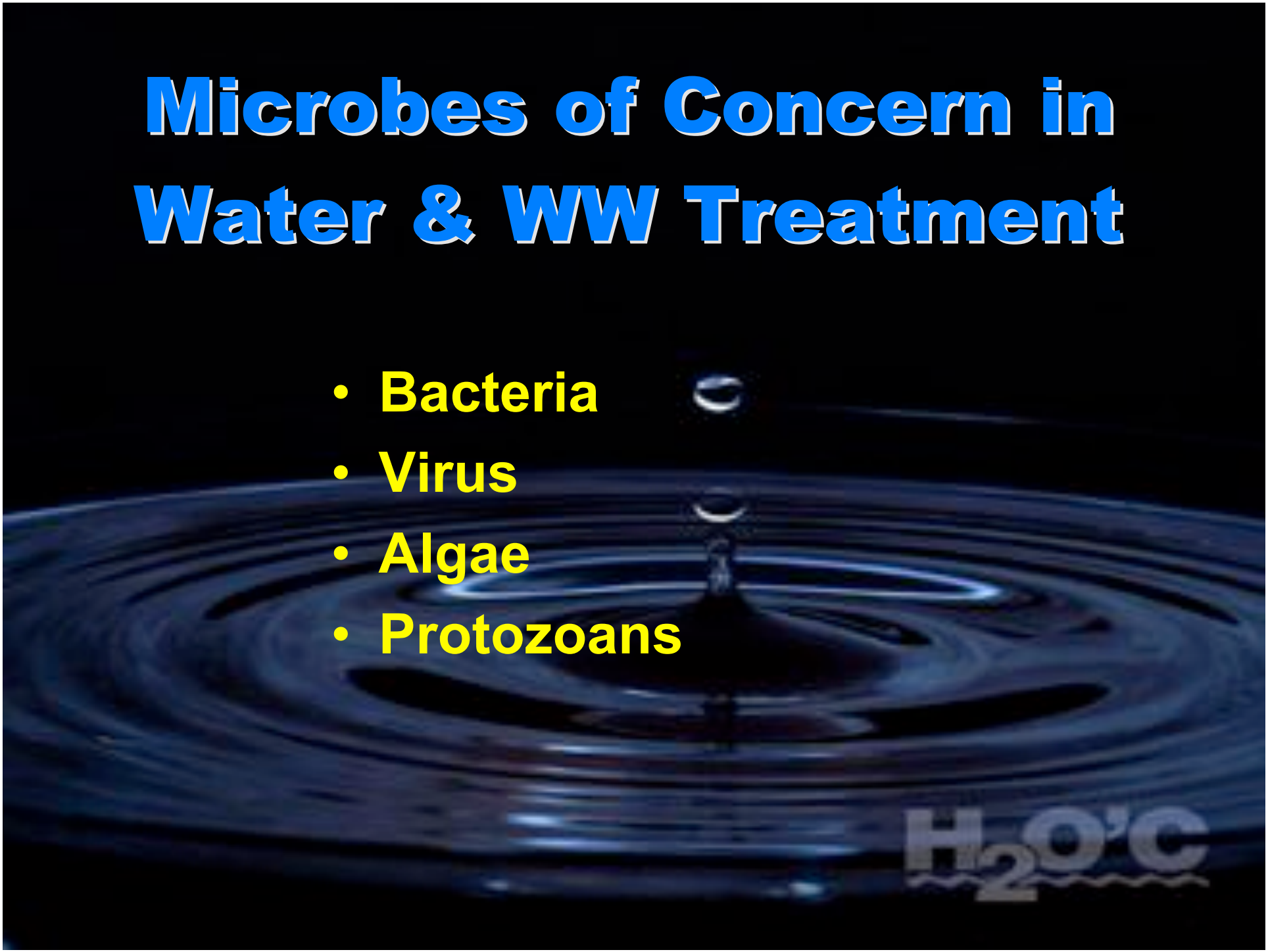




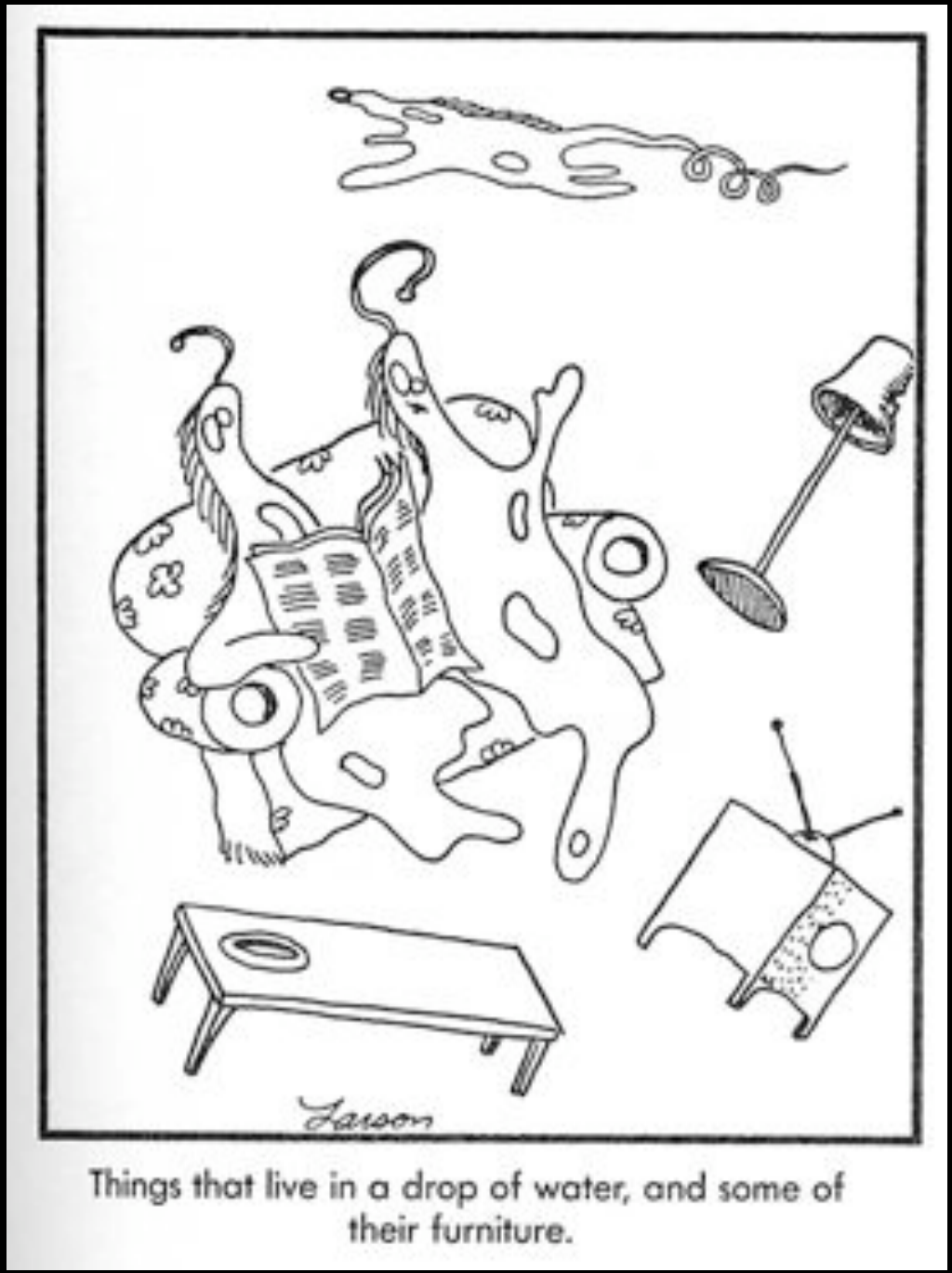




Microbes of Concern in Water & WW Treatment



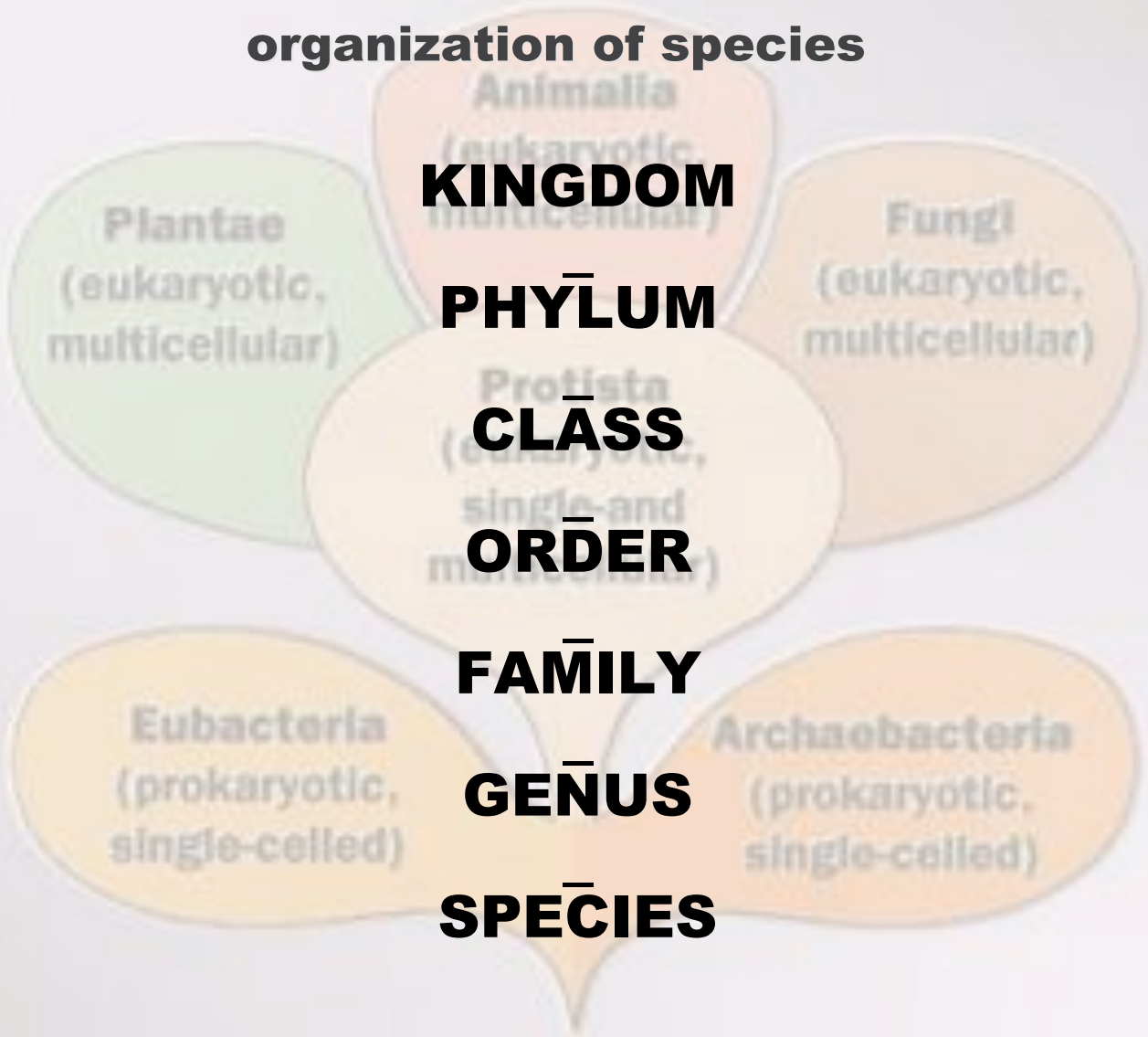
- Bacteria
- Virus
- Algae
- Protozoans



Things that live in a drop of water, and some of their furniture.

Taxonomy

organization of species



KINGDOM

PHY̅LUM

CLA̅SS

ORDER

FAMILY

GENUS

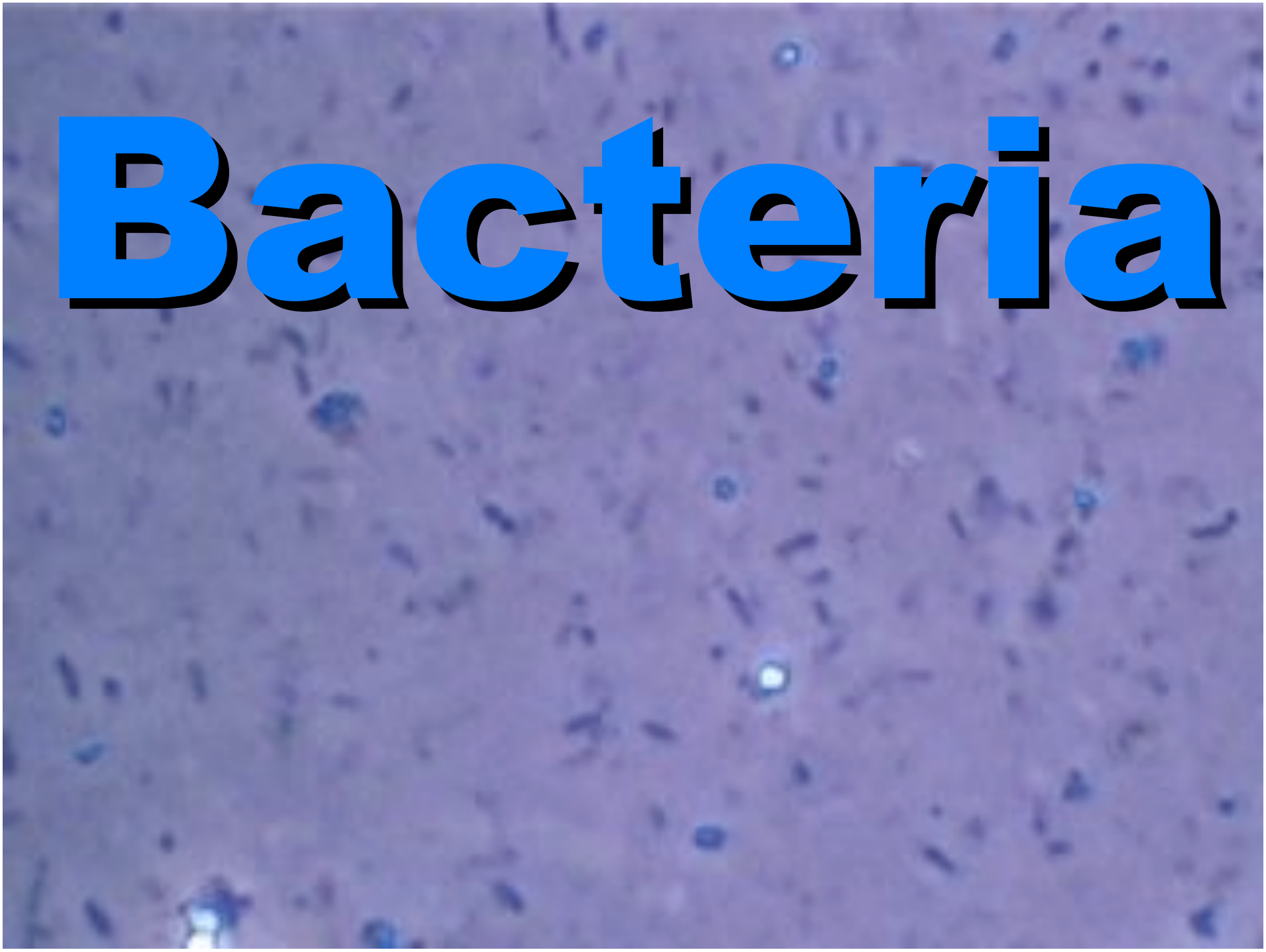
SPE̅CIES

Kings Play Chess On Fine Glass Stools

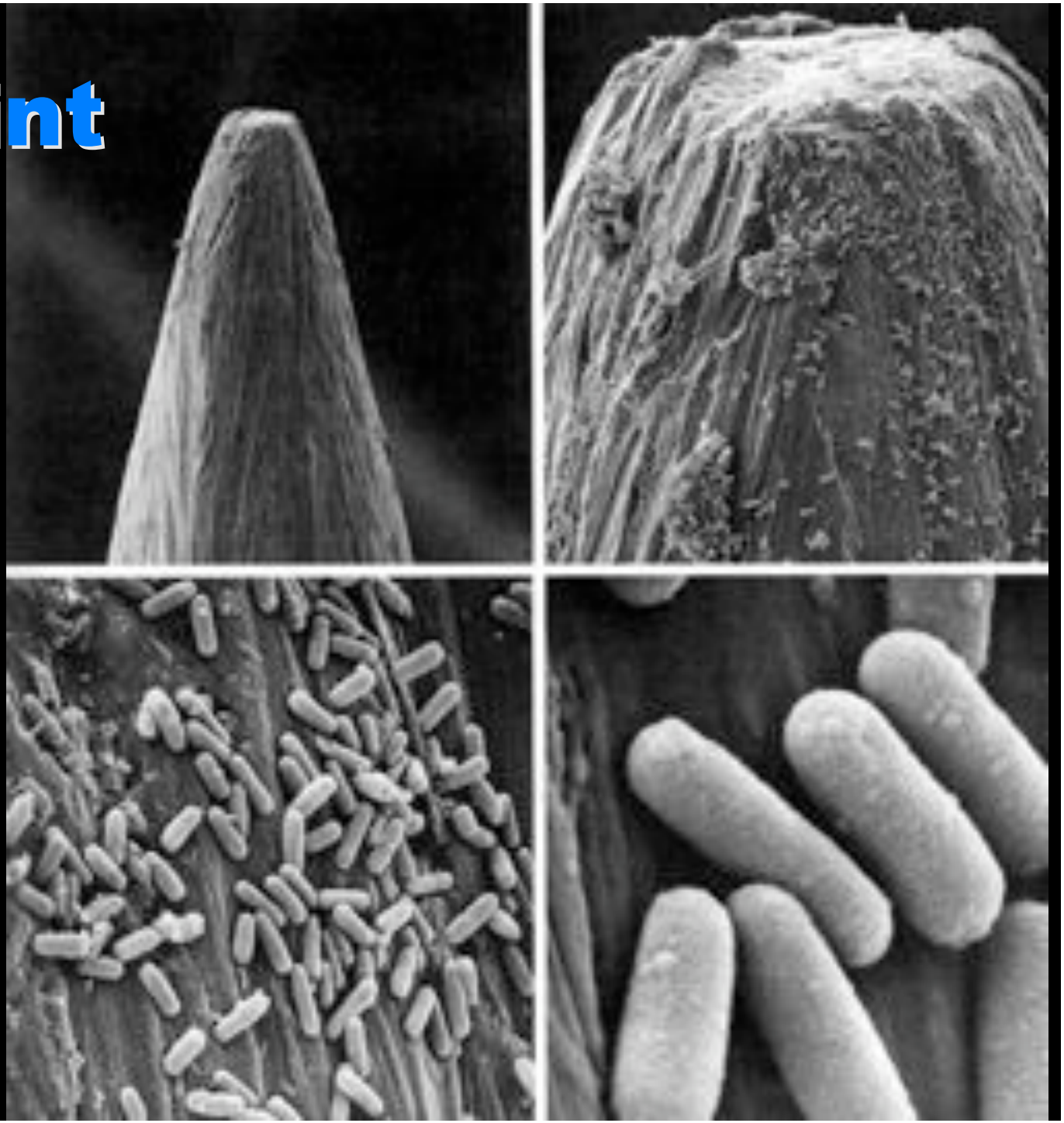
Five Kingdoms

Animals **Plants** **Fungi** **Monera** (bacteria) **Protoctists** (algae, protozoans)

Viruses are not typically considered to be living organisms due to their inability to replicate without a host cell


Bacteria

Pin Point



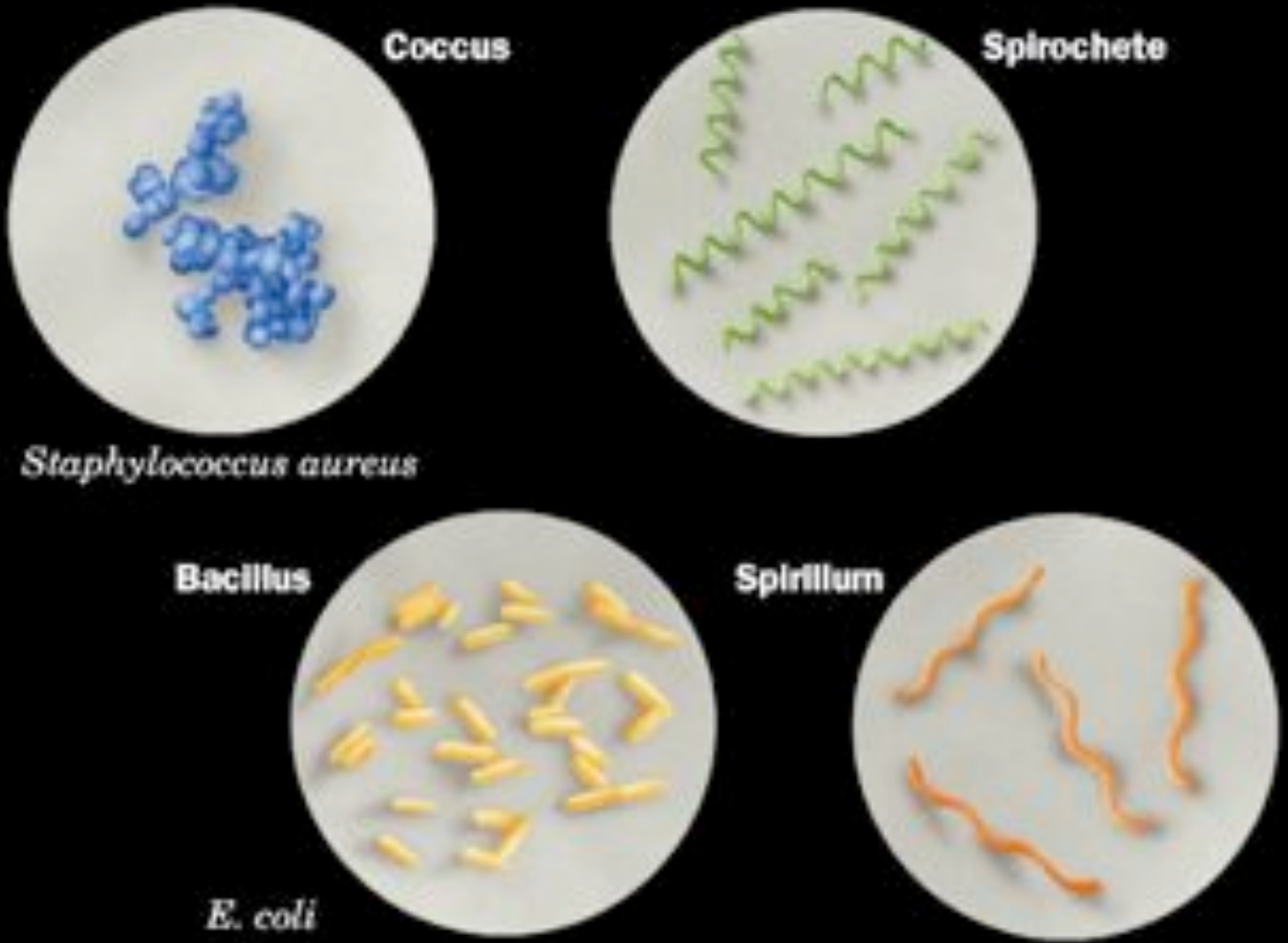
Classification of Bacteria

Bergey's Manual

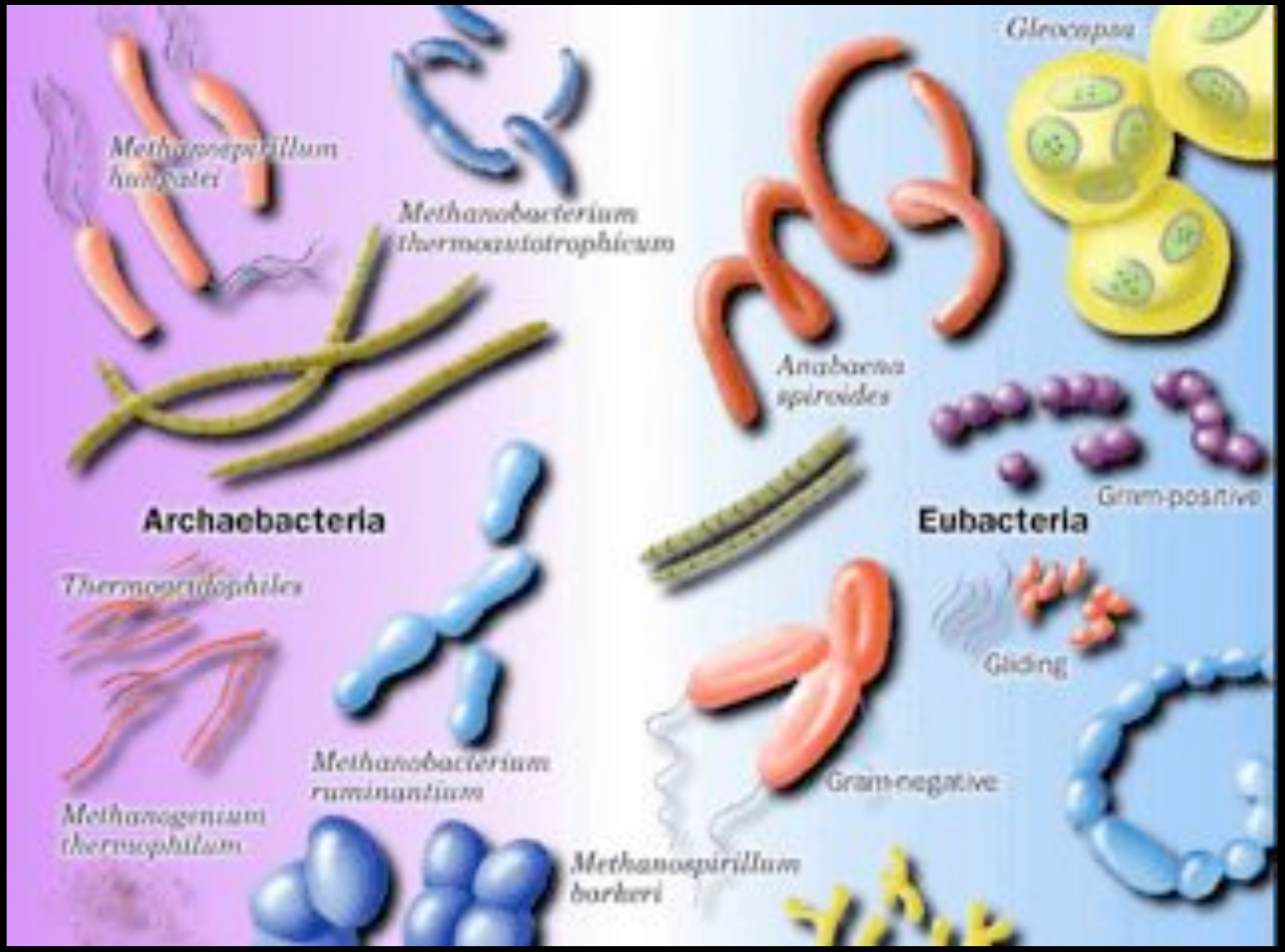
- Archaebacteria
  - Aerobic
  - Anaerobic
- Eubacteria
- Autotrophic bacteria
  - Phototrophic
    - Purple bacteria
    - Green bacteria
  - Chemotrophic
    - Nitrifiers
    - Sulfur oxidizers
    - Fe / Mn oxidizers
    - Methane oxiders
- Heterotrophic bacteria
  - Gram-negative
    - Aerobic
    - Facultatively anaerobic
    - Anaerobic
  - Gram-positive
  - Mycobacteria
- Bacteria with complex structures
  - Actinomycetes
  - Stalked and budding bacteria
  - Sheathed bacteria
  - Gliding and creeping bacteria
  - Spirochaetes
  - Mycoplasmas

Morphology

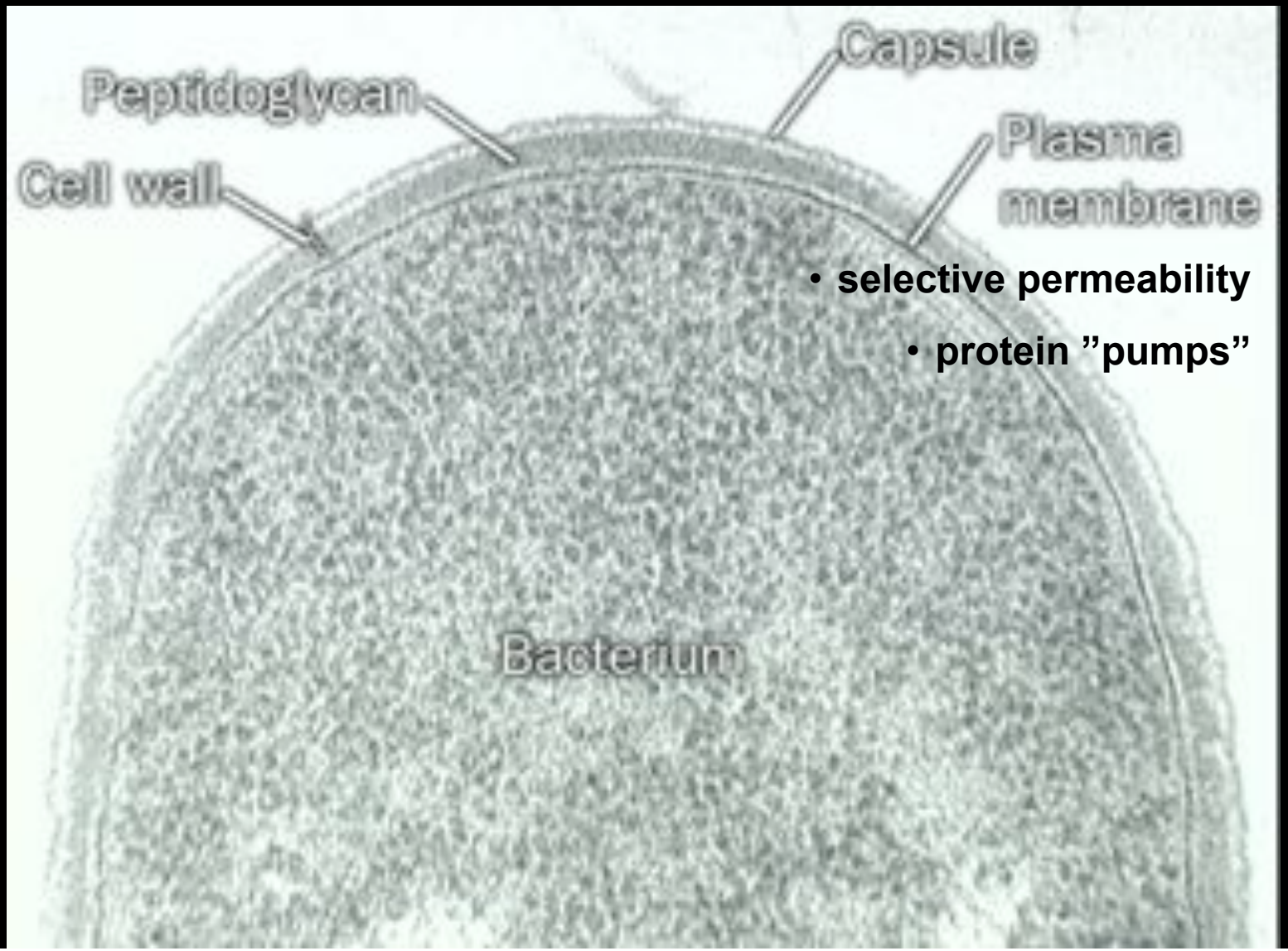
Fancy Word for 'Shape'



Types of Bacteria

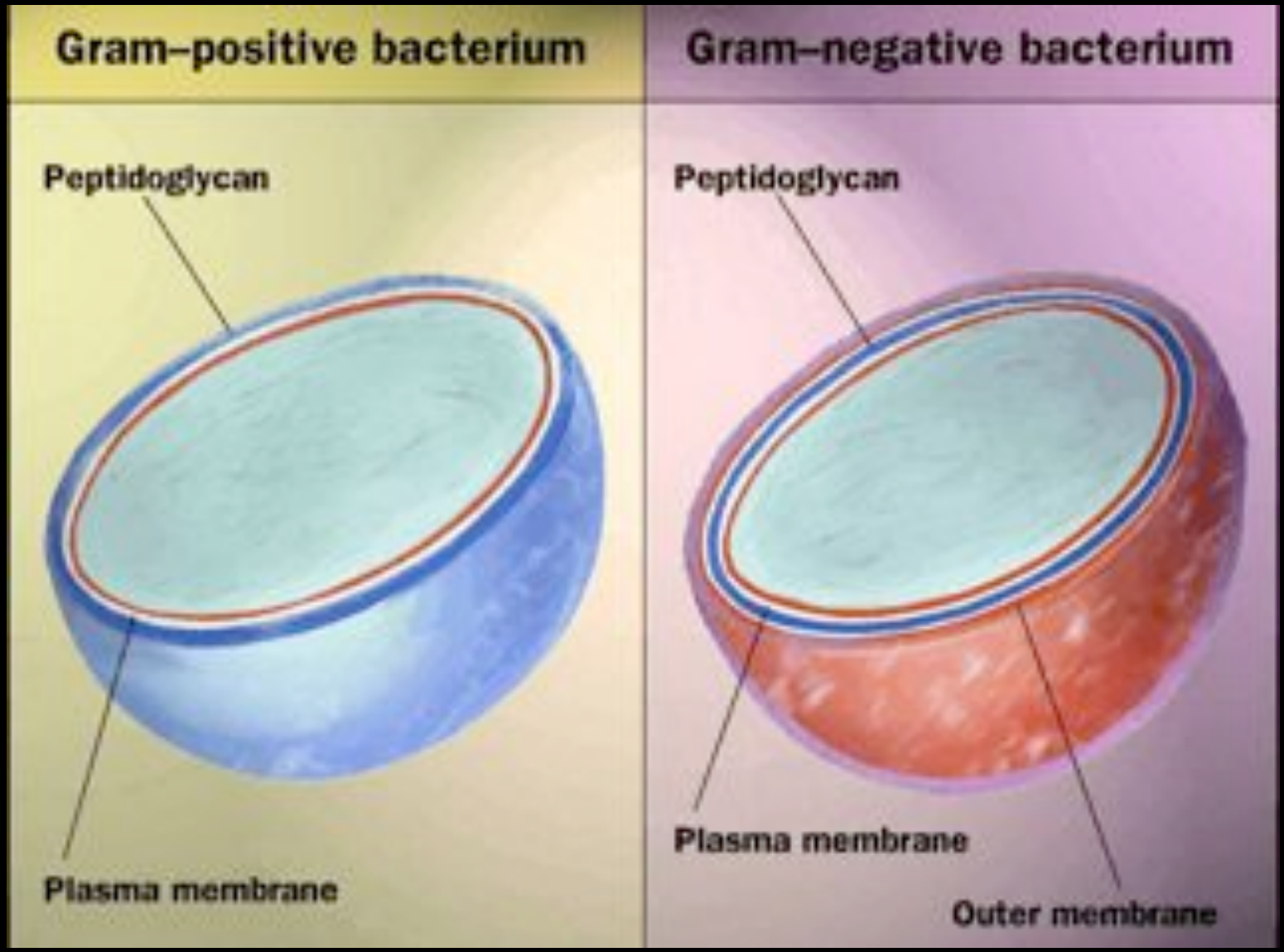


Cell Wall



- selective permeability
- protein ”pumps”

Gram-positive/negative



Food

Autotrophs (self-nourishing)

- require water, CO_2 , inorganic salts, energy source

Heterotrophs

- saprophytic—absorb nutrients through cell membrane
- holozoic—eat, digest, and absorb particulate food

Oxygen

Aerobes

utilize oxygen in respiration

Obligate Anerobes

quickly killed by oxygen

Facultative Anerobes

can take it or leave it

Reproduction

Asexually (fission)

a cell divides into two cells

Kinetics (speed)

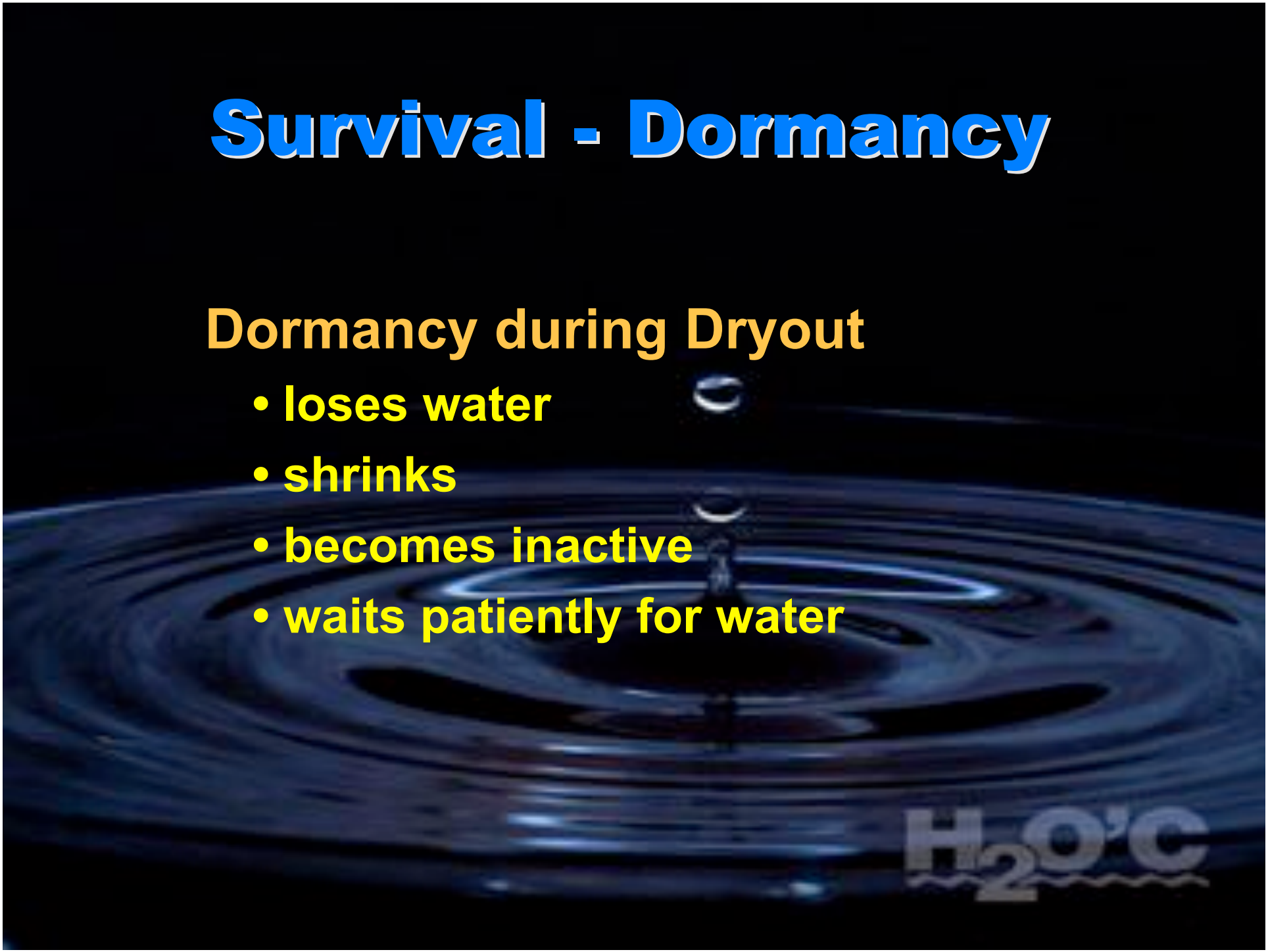
Cells can divide every 20 minutes

One cell ⊥ 8 hours ⊥ 12,000,000 cells

Inhibitors

- lack of food
- accumulation of waste products
- predators

Survival - Dormancy

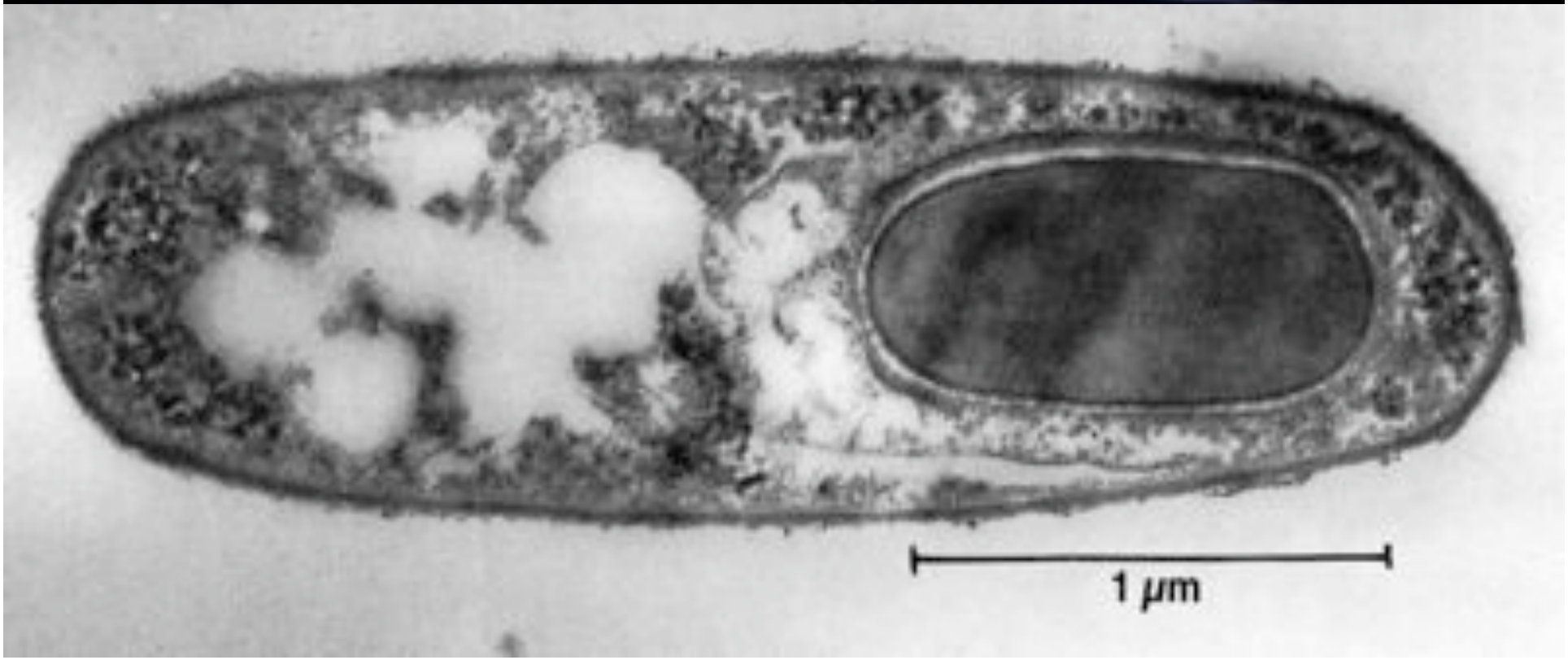


Dormancy during Dryout

- loses water
- shrinks
- becomes inactive
- waits patiently for water

Survival - Spores

- cell retreats in times of unfavorable conditions (dryness, temp, disinfectant)
- forms new, thicker cell wall within old one
- when favorable conditions reappear, spore absorbs water, breaks out of inner shell, returns to normal
- Anthrax bacilli can survive 30 years in spore form
- most pathogenic (disease-causing) bacteria do not form spores

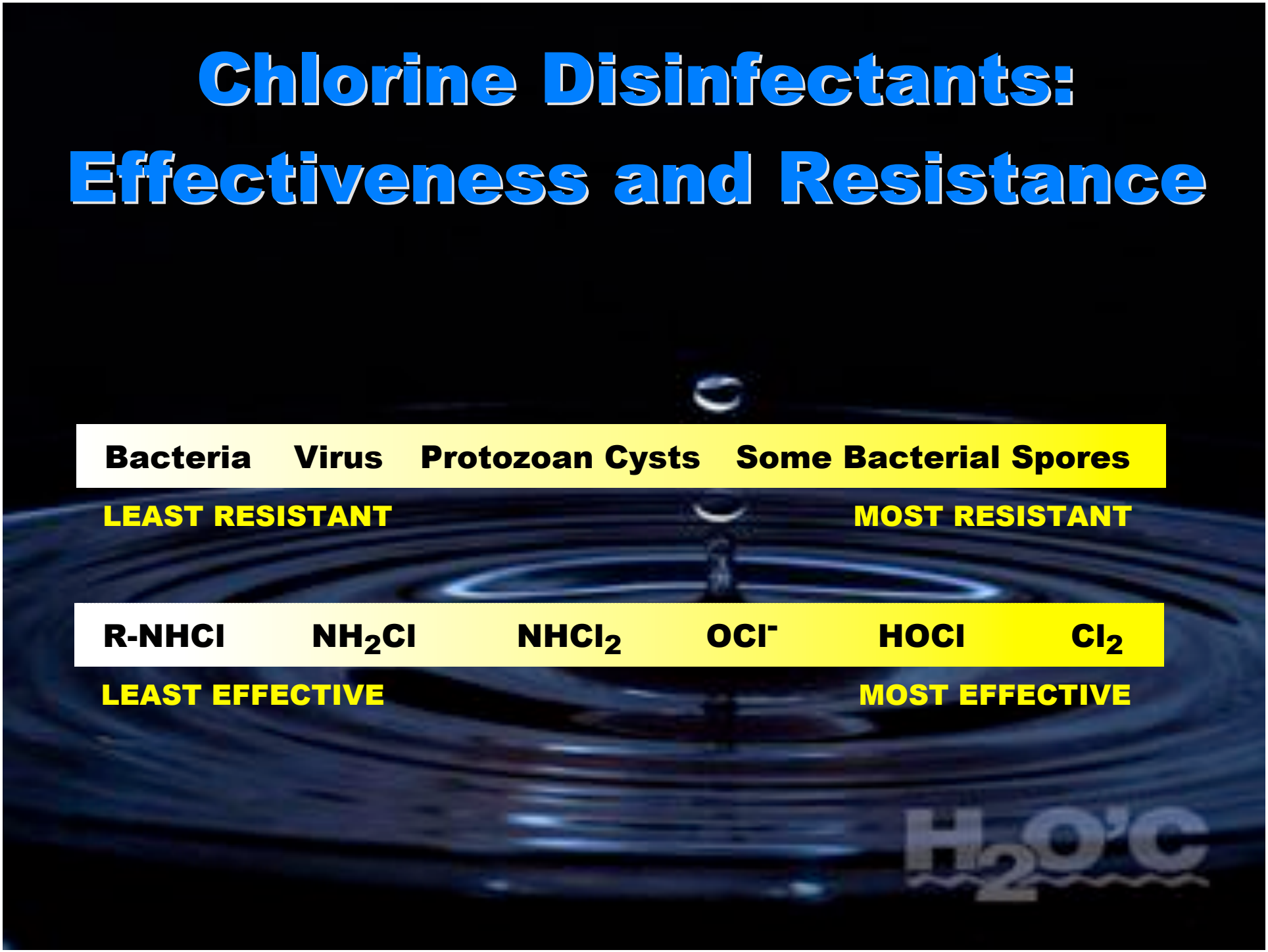


Ailments
Associated with Bacteria

typhoid fever	⊥	Bacillus typhosus
diarrhea	⊥	Escherichia coli
Legionnaire's disease	⊥	Legionella
Leptospirosis	⊥	Leptospirea
salmonellosis, paratyphoid	⊥	Salmonella
bacillary dysentary (Shigellosis)	⊥	Shigella
cholera	⊥	Vibrio cholerae
plague	⊥	Yersinia

There are over 3,000 species of bacteria;
only a handful are pathogenic (disease-causing)

Chlorine Disinfectants: Effectiveness and Resistance



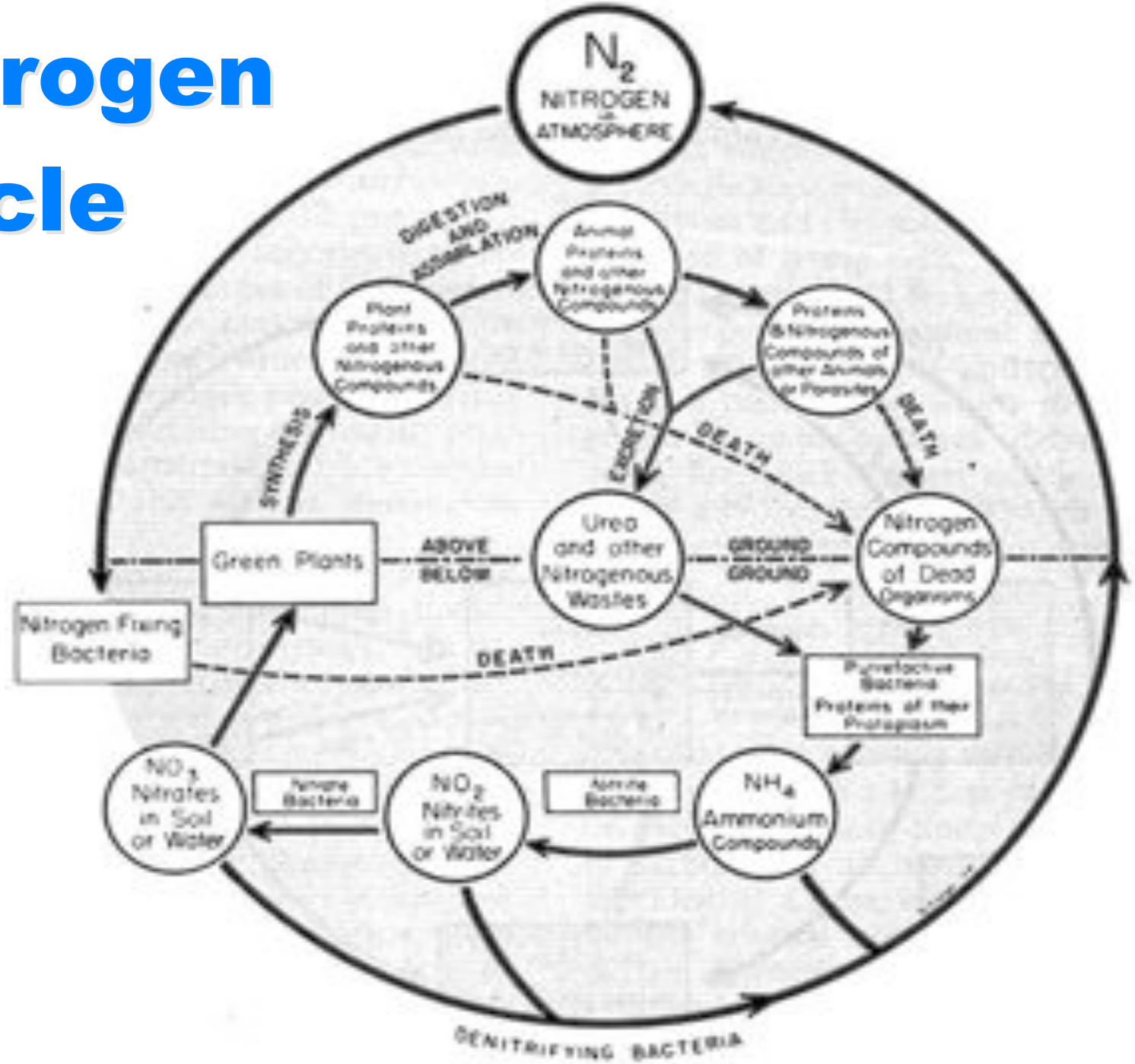
Bacterial Jobs

Fermentation: enzymatic anaerobic breakdown of carbohydrates

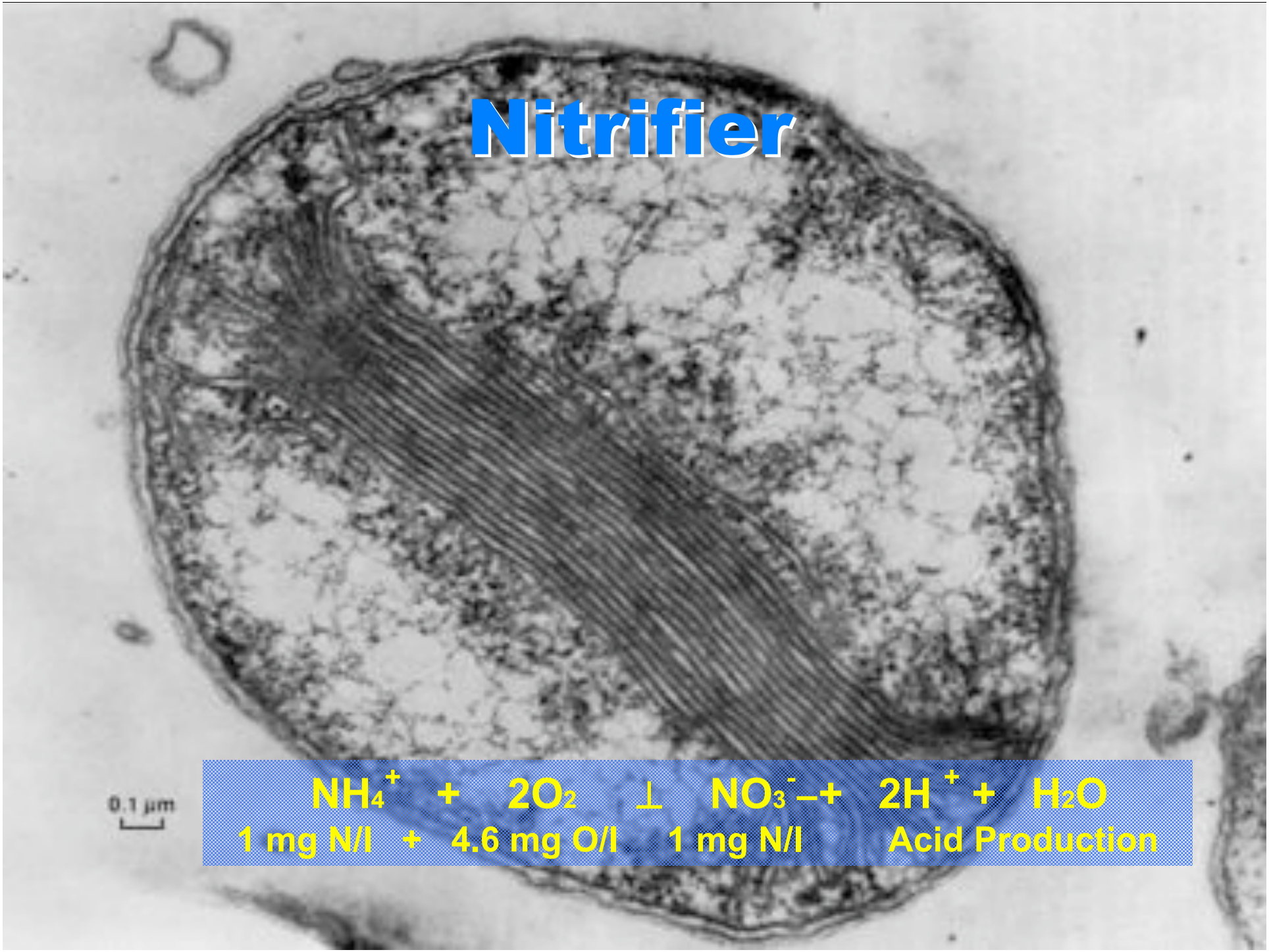
Putrefaction: enzymatic anaerobic breakdown of proteins and amino acids

- Stench—nitrogen-and sulfur-containing compounds produced during putrefaction

Nitrogen Cycle



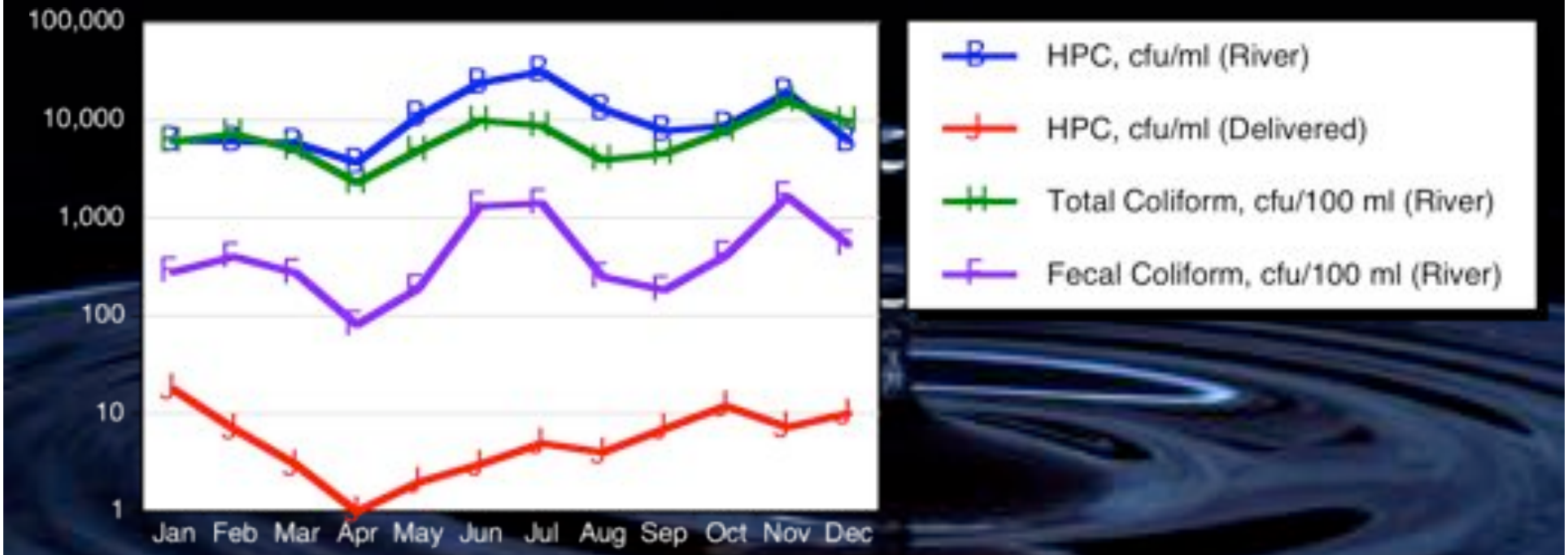
Nitrifier



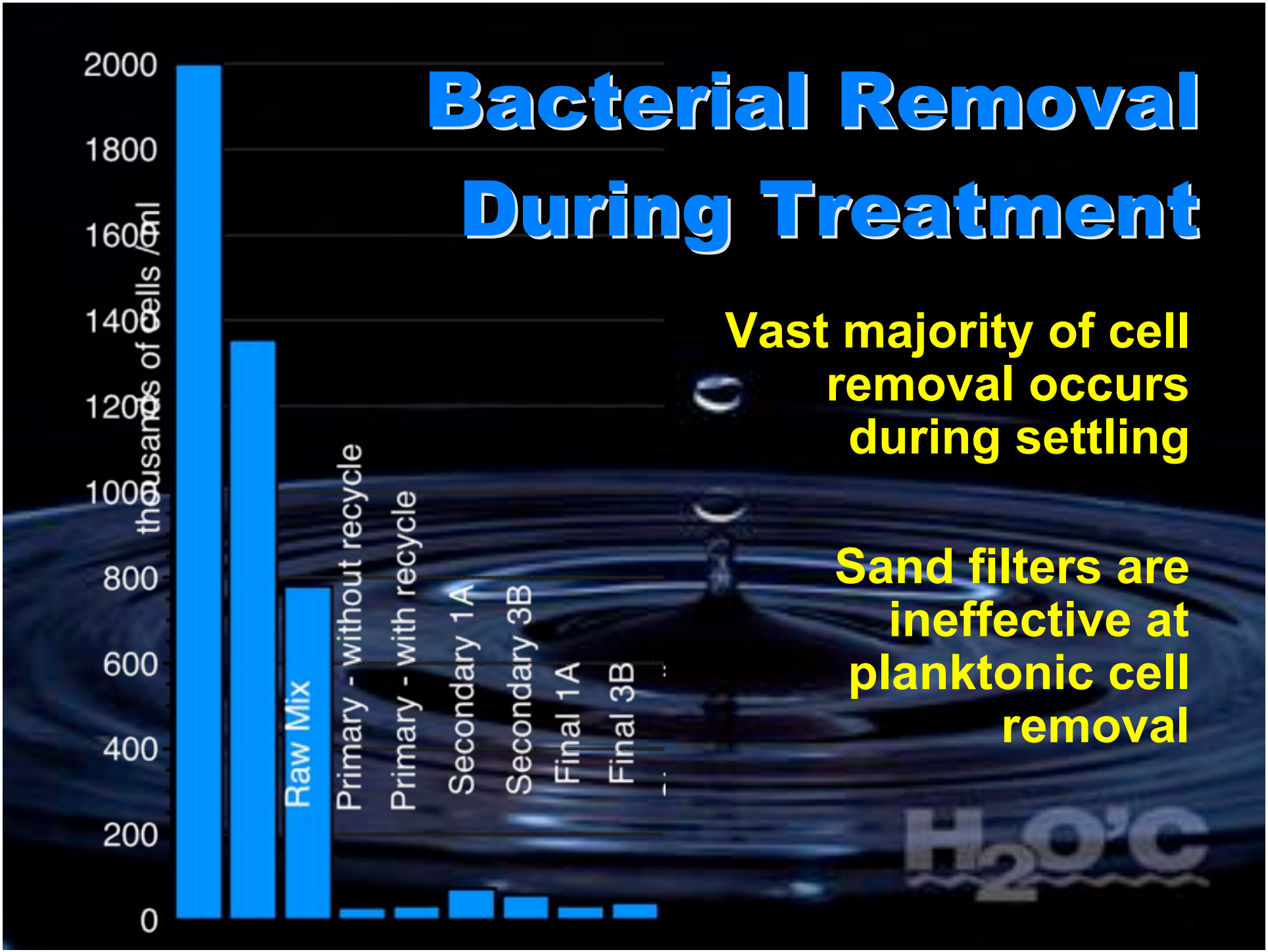
$$NH_4^+ + 2O_2 \perp NO_3^- + 2H^+ + H_2O$$

1 mg N/l + 4.6 mg O/l 1 mg N/l Acid Production

HPC & Coliform

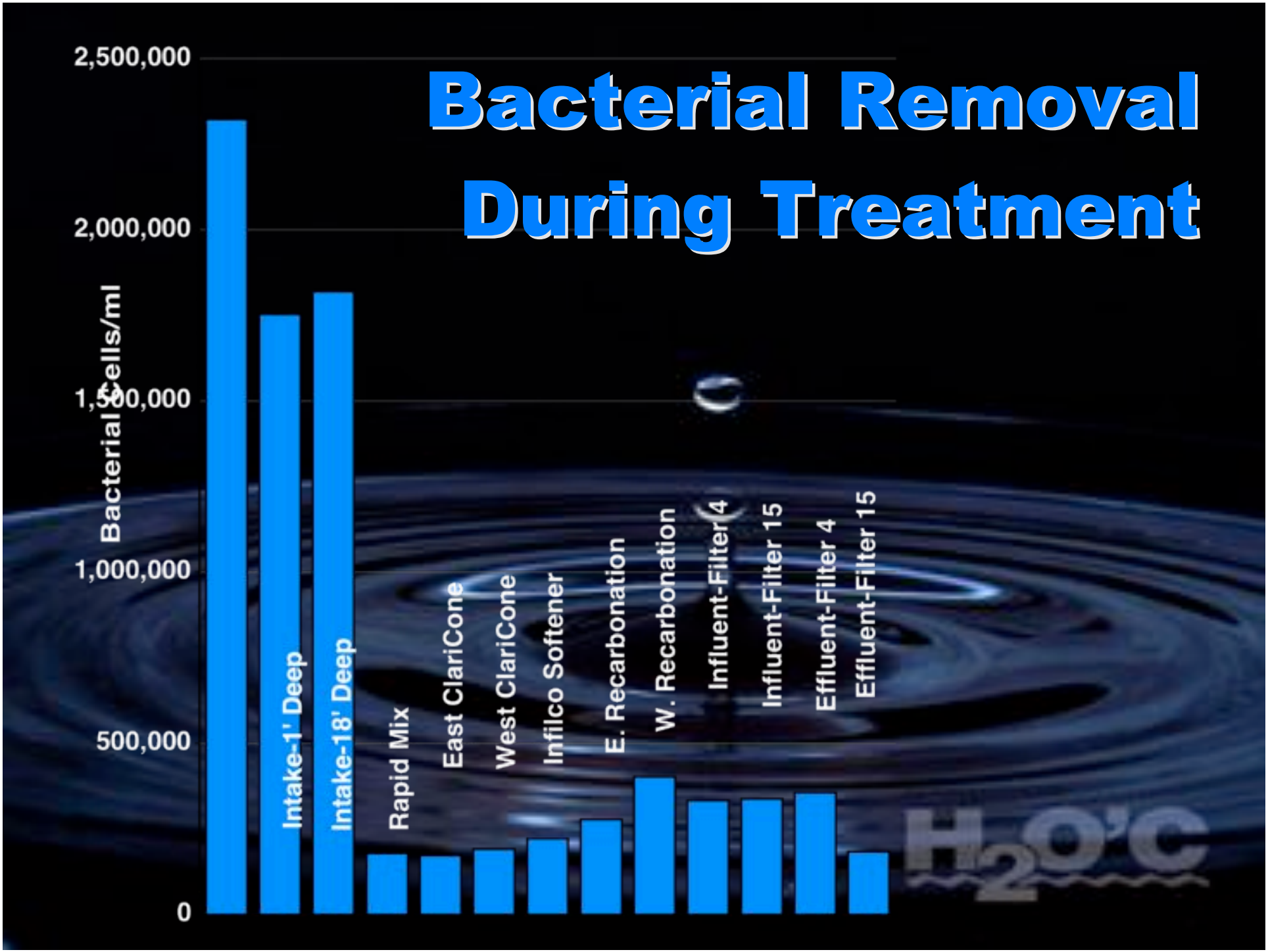


Bacterial Removal During Treatment

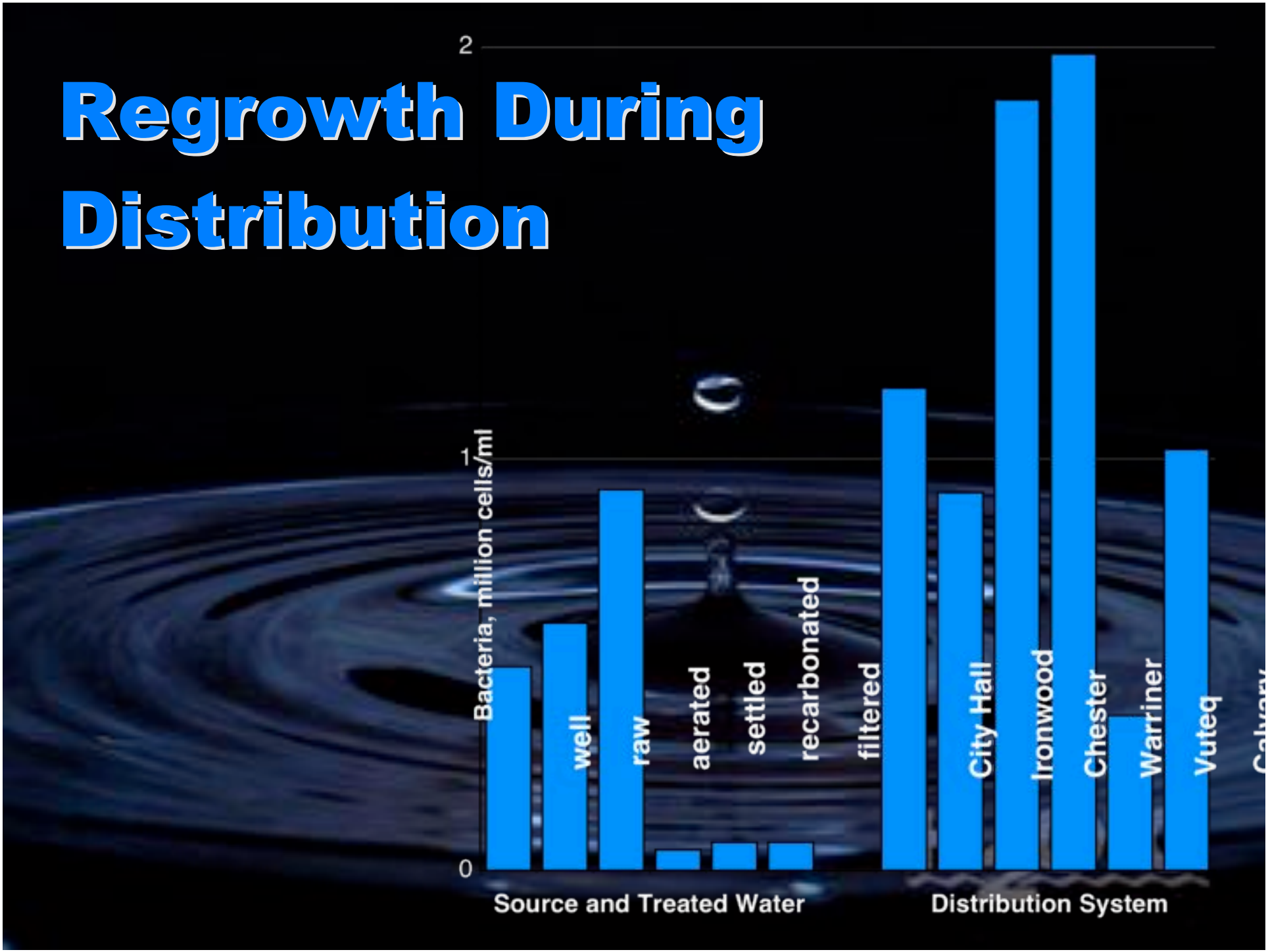


Vast majority of cell removal occurs during settling

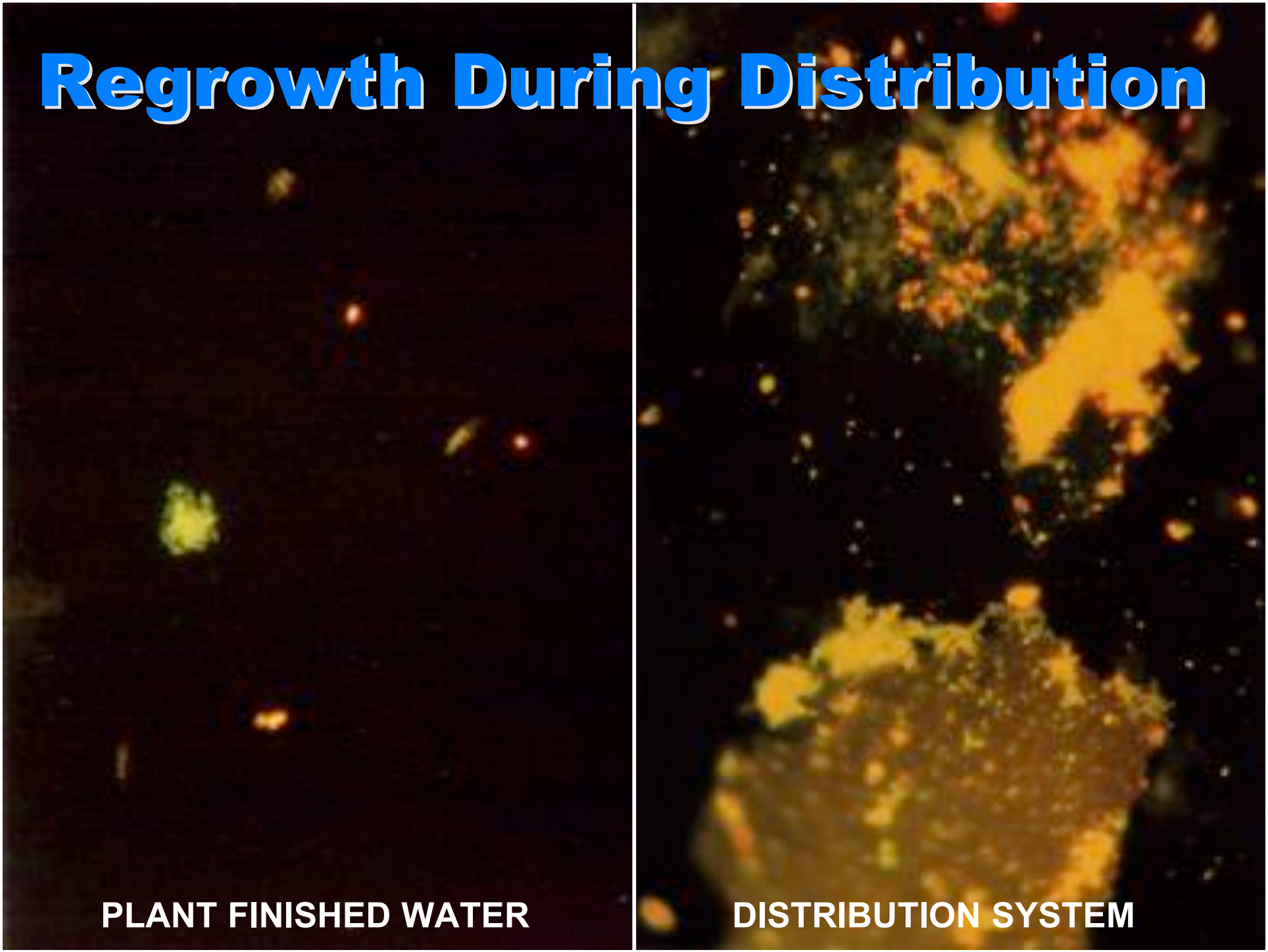
Sand filters are ineffective at planktonic cell removal


Bacterial Removal During Treatment
Bacterial Cells/ml
2,500,000
2,000,000
1,500,000
1,000,000
500,000
0
Intake-1' Deep
Intake-18' Deep
Rapid Mix
East ClariCone
West ClariCone
Infilco Softener
E. Recarbonation
W. Recarbonation
Influent-Filter 4
Influent-Filter 15
Effluent-Filter 4
Effluent-Filter 15
H2O'C

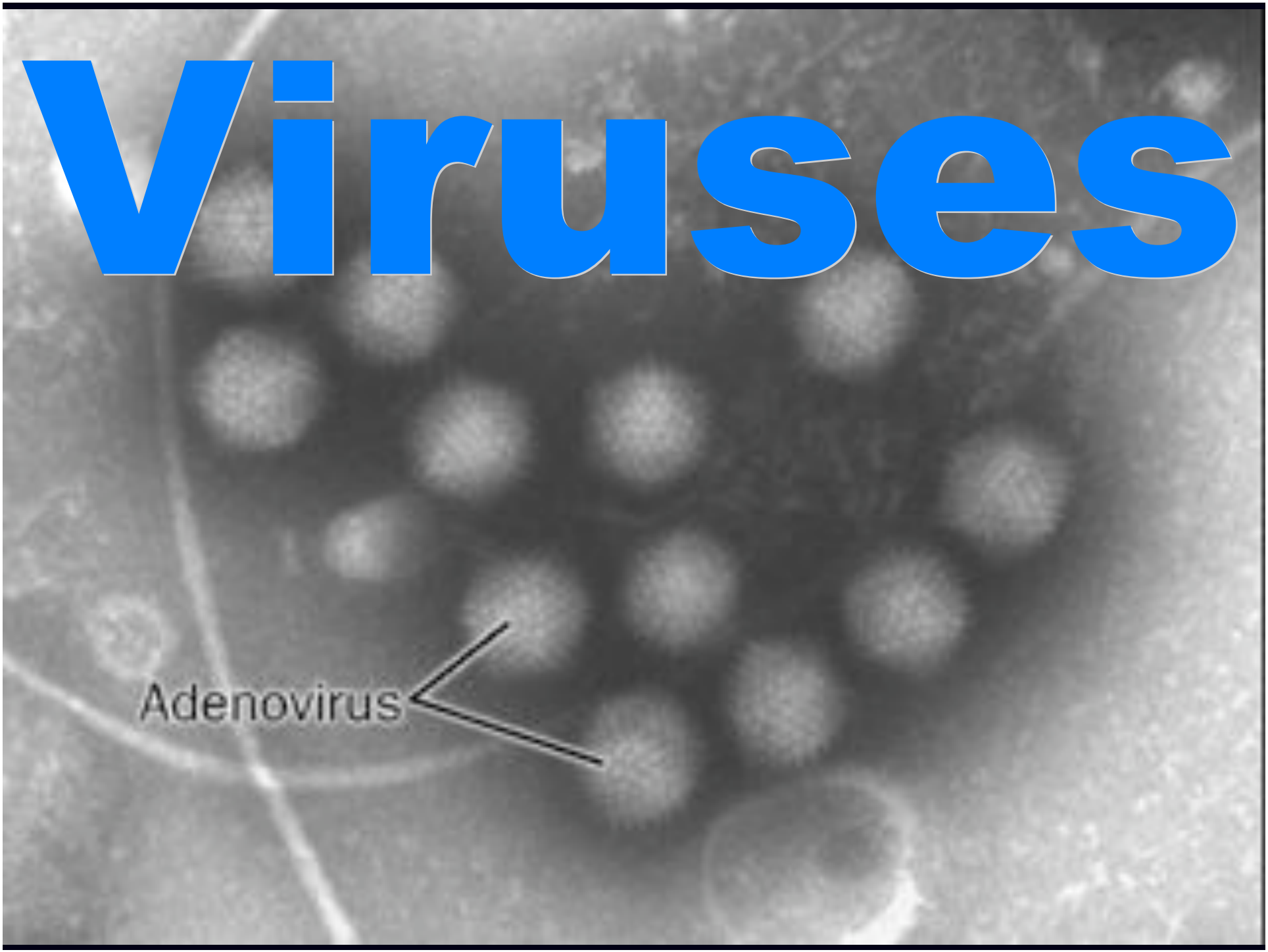
Regrowth During Distribution



Regrowth During Distribution



Viruses



Viruses

	Virus	Nucleic Acid Type	Shape	Size (nm) (or diameter × length)
Animal Viruses	Vaccinia	DNA		230 × 300
	Mumps	RNA		150 × 300
	Herpes	DNA		100 × 200
	Influenza	RNA		80 × 120
	Adenovirus	DNA		70 × 90
	Poliovirus	RNA		28
Plant Viruses	Wound tumor	RNA		55 × 60
	Tobacco mosaic	RNA		18 × 300
	Potato X	RNA		10 × 500
Bacterial Phages	T phage	DNA		65 × 200
	ϕX174	DNA		25

* Adapted from H. Lechevalier and D. Pramer (1971), *The Microbes*. J. B. Lippincott Co., Philadelphia, Pa., and R. W. Horne (1963), "The Structure of Viruses," *Scientific American*.

Viruses - Plant and Bacterial

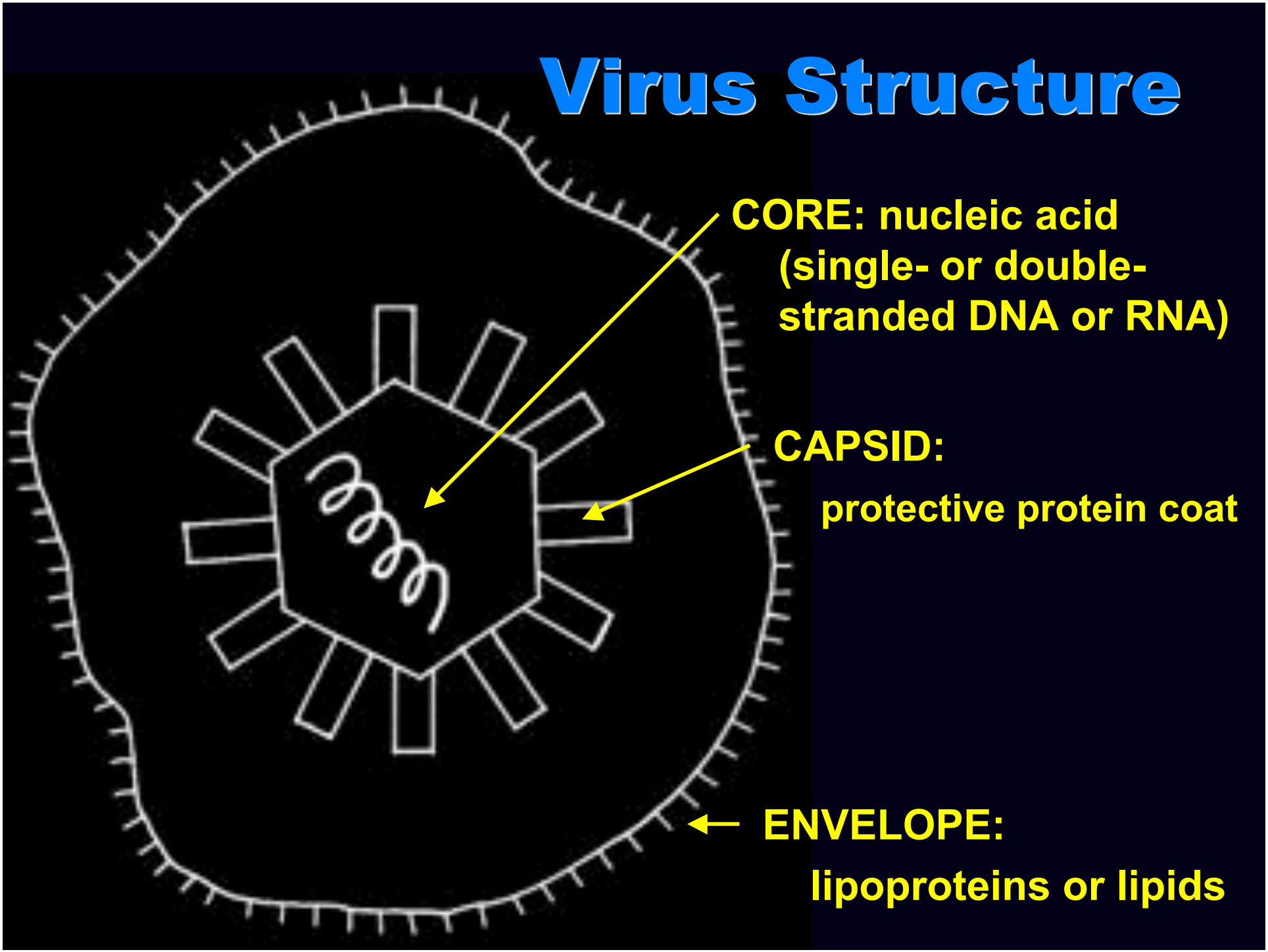
Wound tumor	RNA		55 × 60
Tobacco mosaic	RNA		18 × 300
Potato X	RNA		10 × 500
T phage	DNA		65 × 200
ϕX174	DNA		25

[a] Adapted from H. Lechevalier and D. Pramer (1971), *The Microbes*. J. B. Lippincott Co., Philadelphia, Pa., and R. W. Horne (1963), "The Structure of Viruses," *Scientific American*.

Viruses - Animal

Virus	*Nucleic Acid Type*	*Shape*	*Size (nm) (or diameter × length)*
Vaccinia	DNA		230 × 300
Mumps	RNA		150 × 300
Herpes	DNA		100 × 200
Influenza	RNA		80 × 120
Adenovirus	DNA		70 × 90
Poliovirus	RNA		28

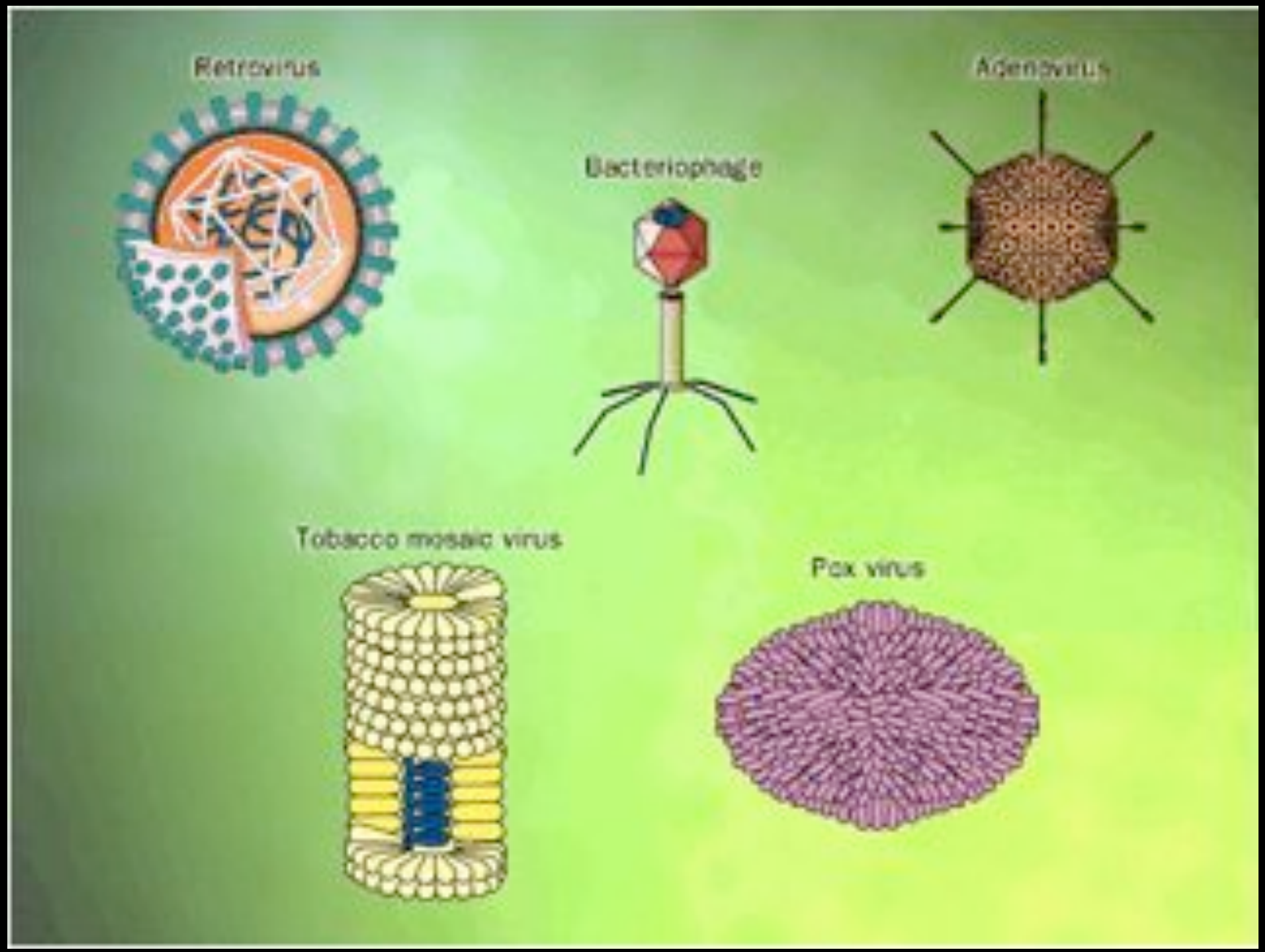
Virus Structure



CORE: nucleic acid (single- or double-stranded DNA or RNA)

CAPSID: protective protein coat

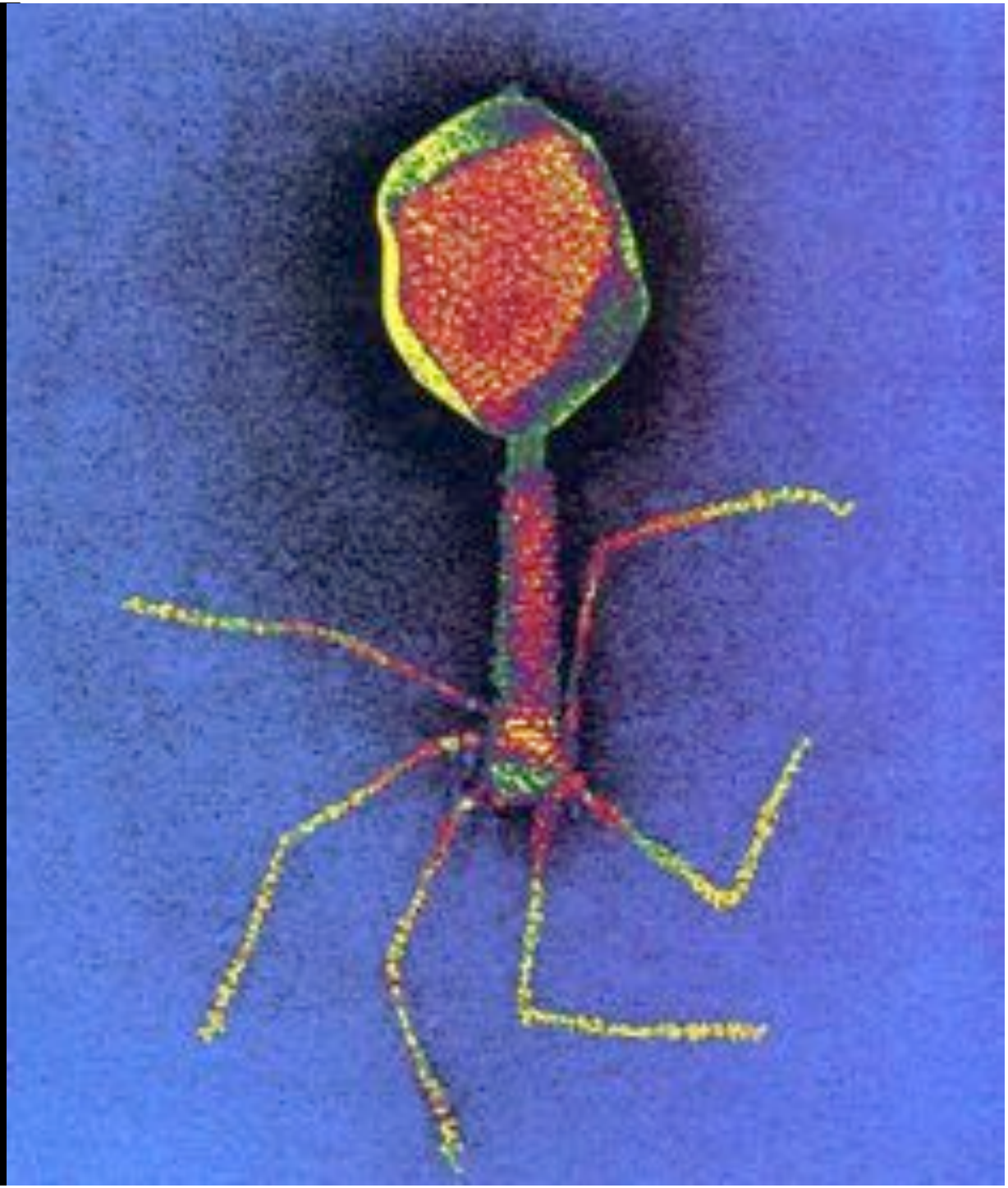
ENVELOPE: lipoproteins or lipids


Retrovirus
Bacteriophage
Adenovirus
Tobacco mosaic virus
Pox virus

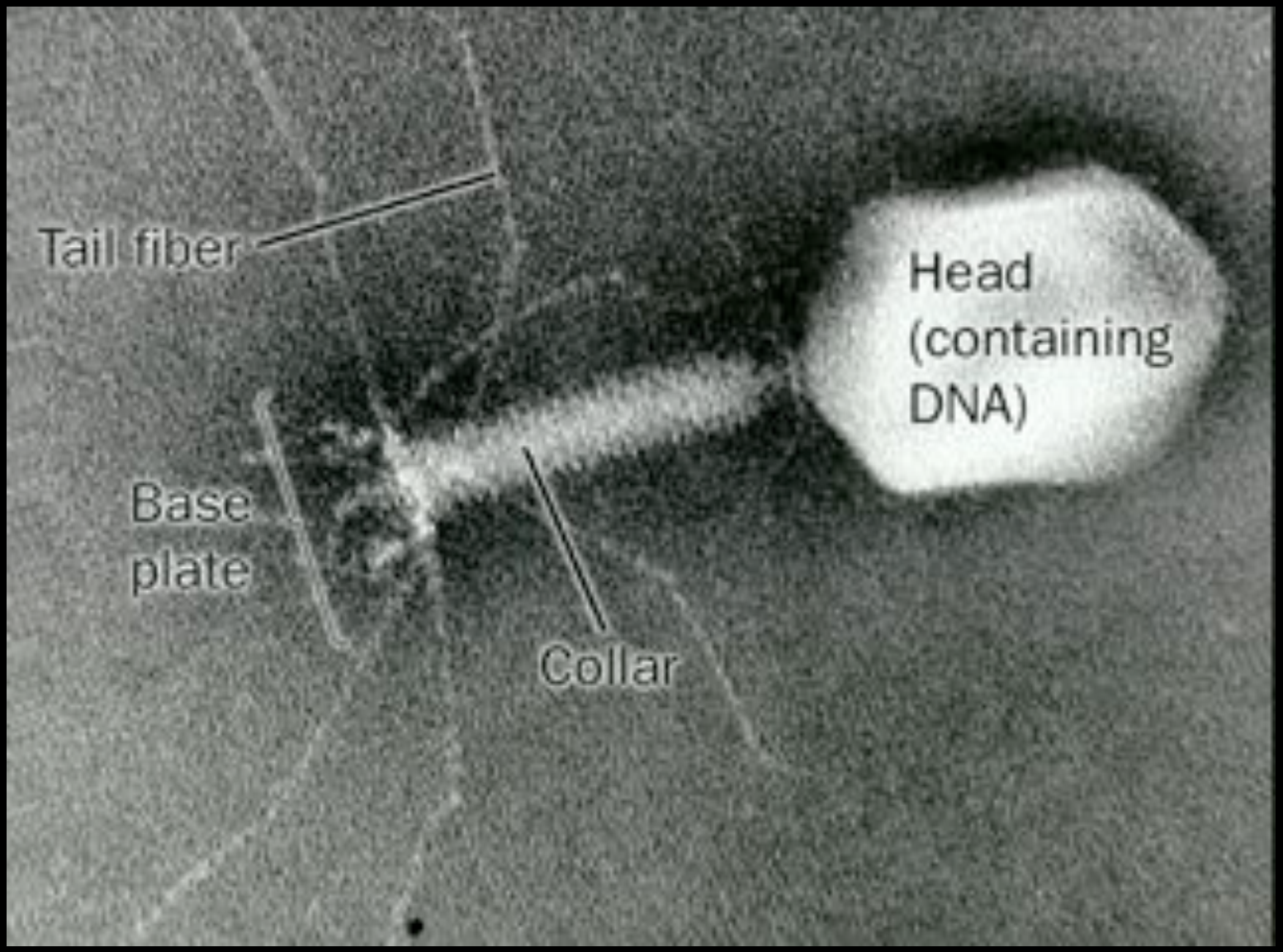
Phage

"one that eats"

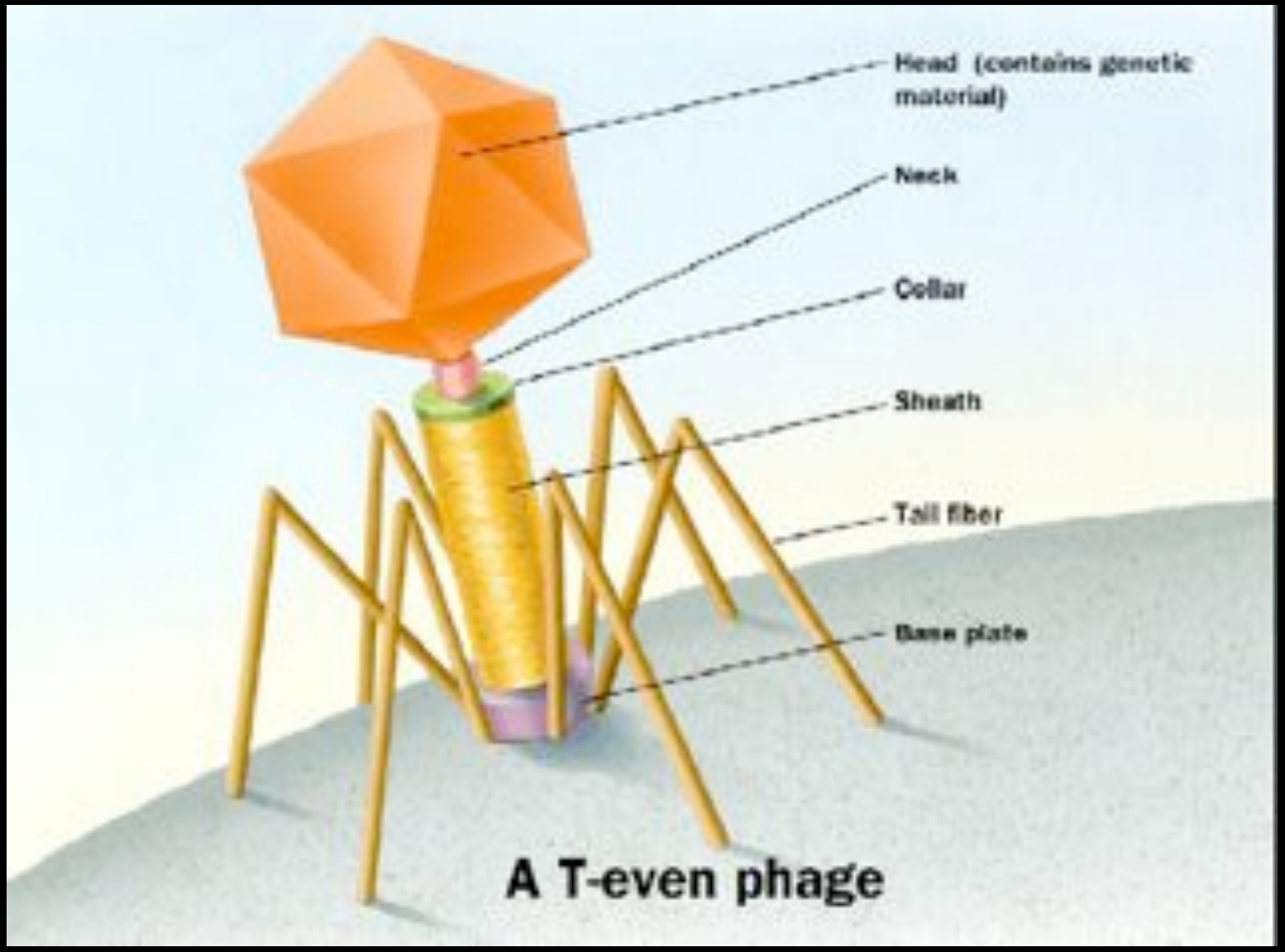
- Head contains DNA
- Legs attach to bacterium
- ≈0.2 μm long

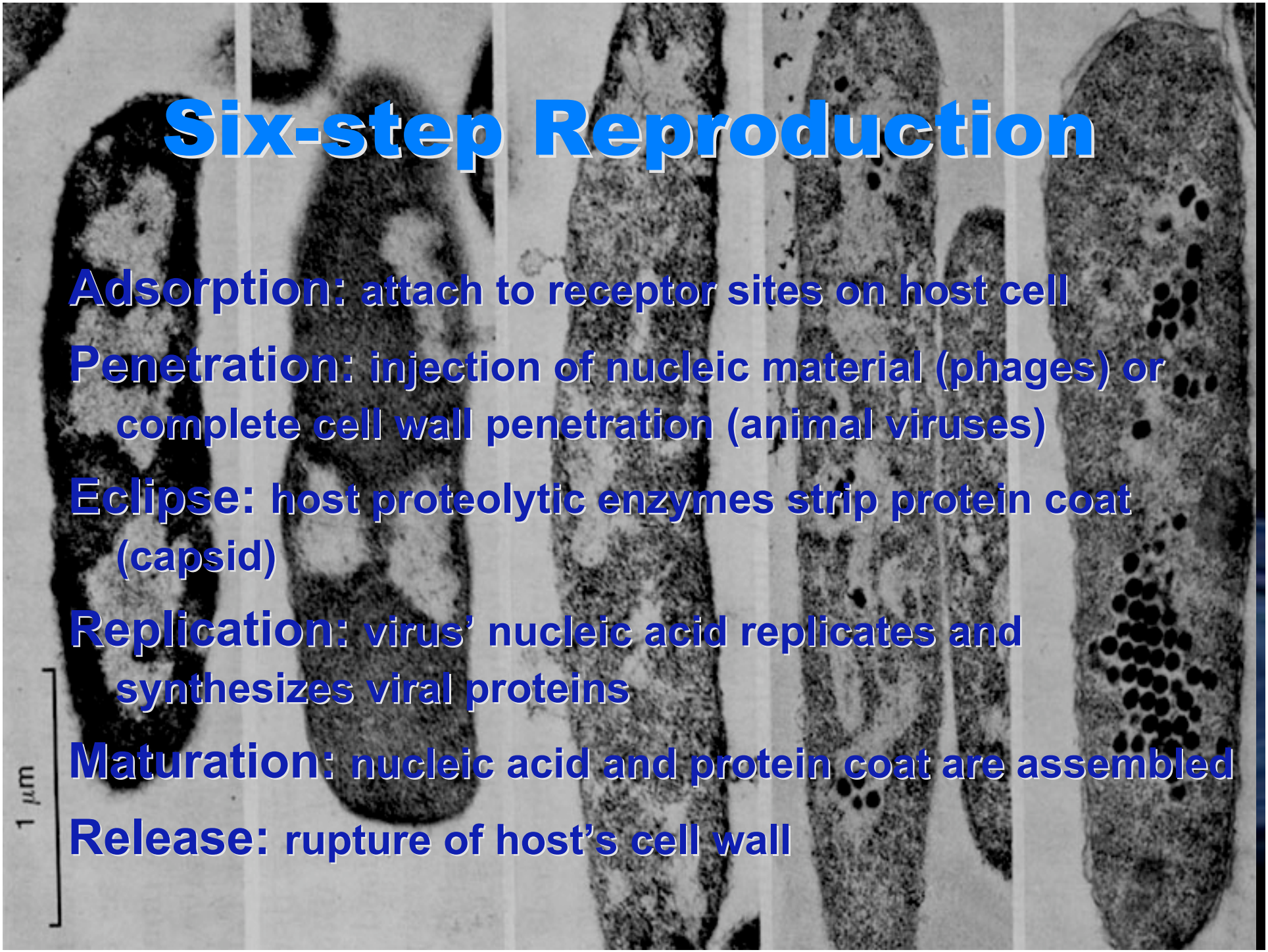


Bacteriophage Micrograph



Bacteriophage





Six-step Reproduction

Adsorption: attach to receptor sites on host cell

Penetration: injection of nucleic material (phages) or complete cell wall penetration (animal viruses)

Eclipse: host proteolytic enzymes strip protein coat (capsid)

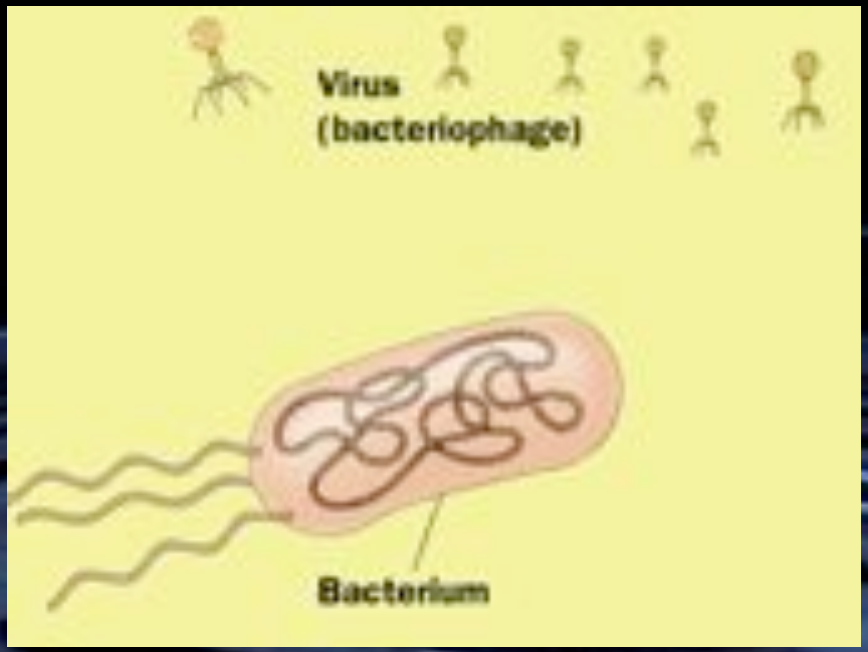
Replication: virus' nucleic acid replicates and synthesizes viral proteins

Maturation: nucleic acid and protein coat are assembled

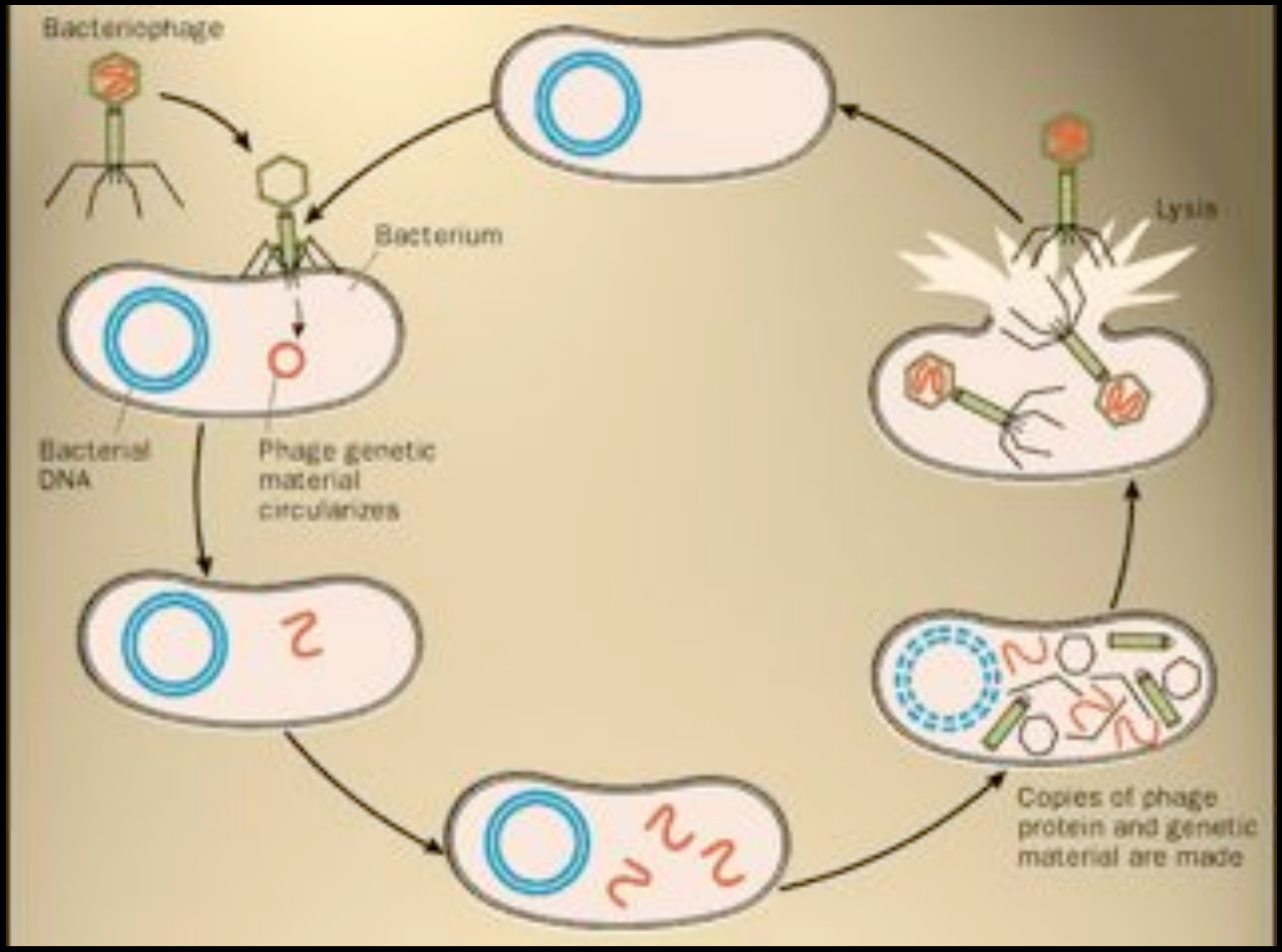
Release: rupture of host's cell wall

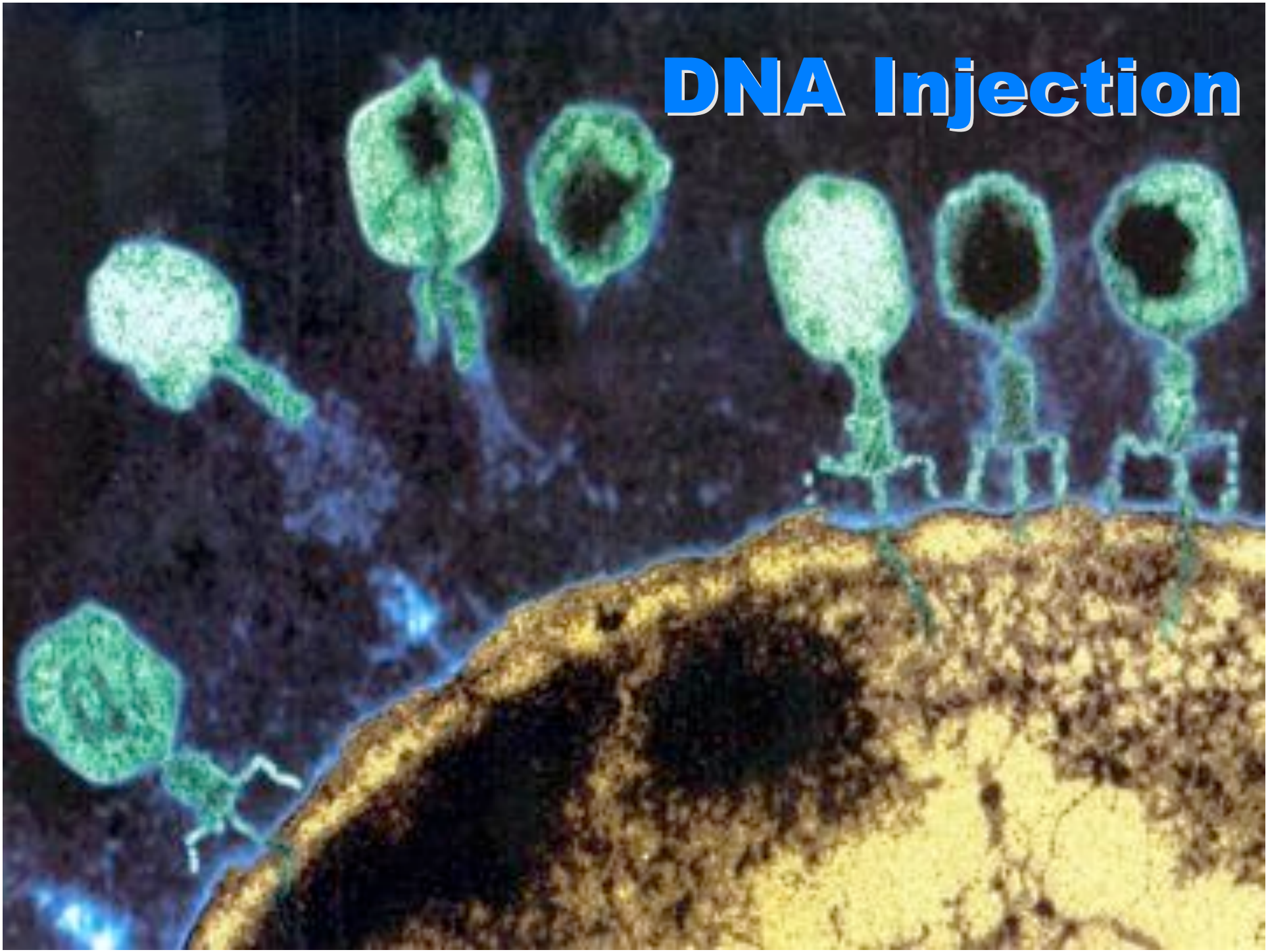
Bacteriophage Reproduction

Phage Invaders

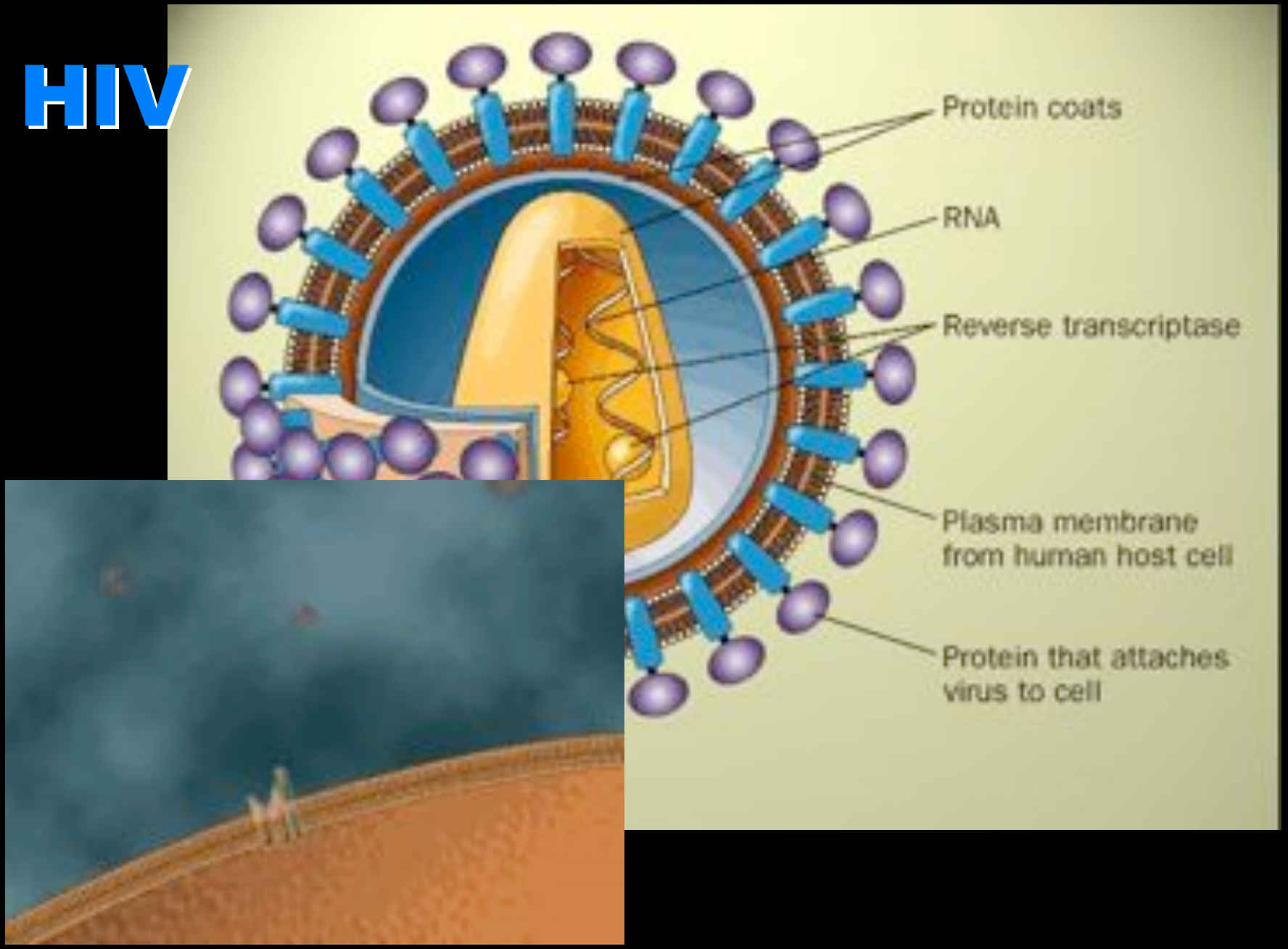


Bacteriophage Cycle




DNA Injection

HIV

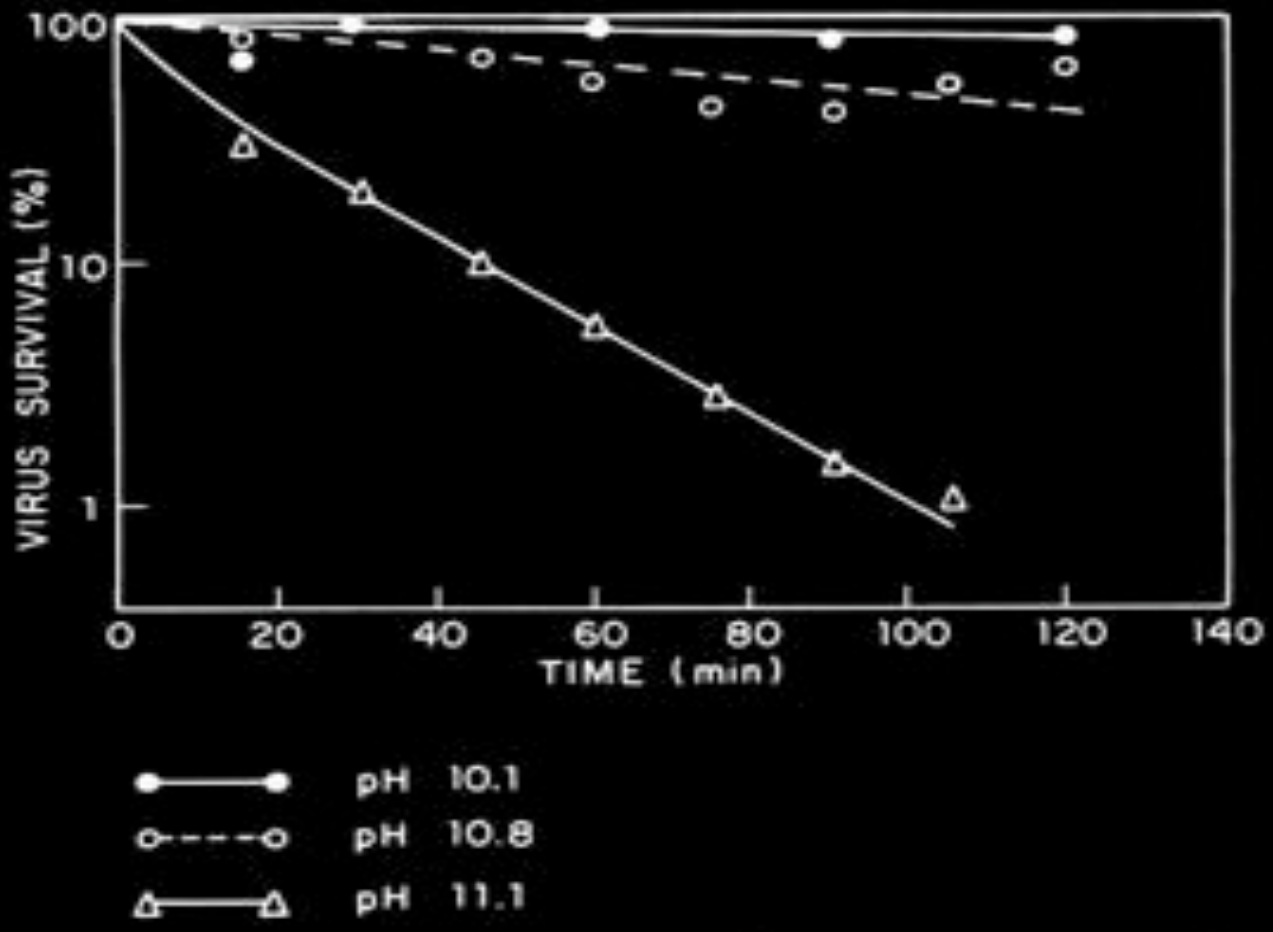

Protein coats
RNA
Reverse transcriptase
Plasma membrane from human host cell
Protein that attaches virus to cell

Ailments Associated with Viruses

Polio (Poliovirus)
Meningitis (Coxsackievirus)
Conjunctivitis (Andenovirus)
Meningitis, epidemic exanthem, infantile diarrhea (ECHO Virus)
Hepatitis (Hepatitis A and B virus)
Gastroenteritis (Reovirus, Rotavirus, Norwalk agent)

Also: colds, flu, fever, rash, eye infection, respiratory illness, smallpox, measles, mumps, herpes, AIDS, cold sores, warts

Effect of pH on Viruses



Effect of high pH on poliovirus 1 (LSc). From G. Berg et al. (1968), *J. Am. Water Works Assoc.* **60**:193.

Inactivation of Viruses

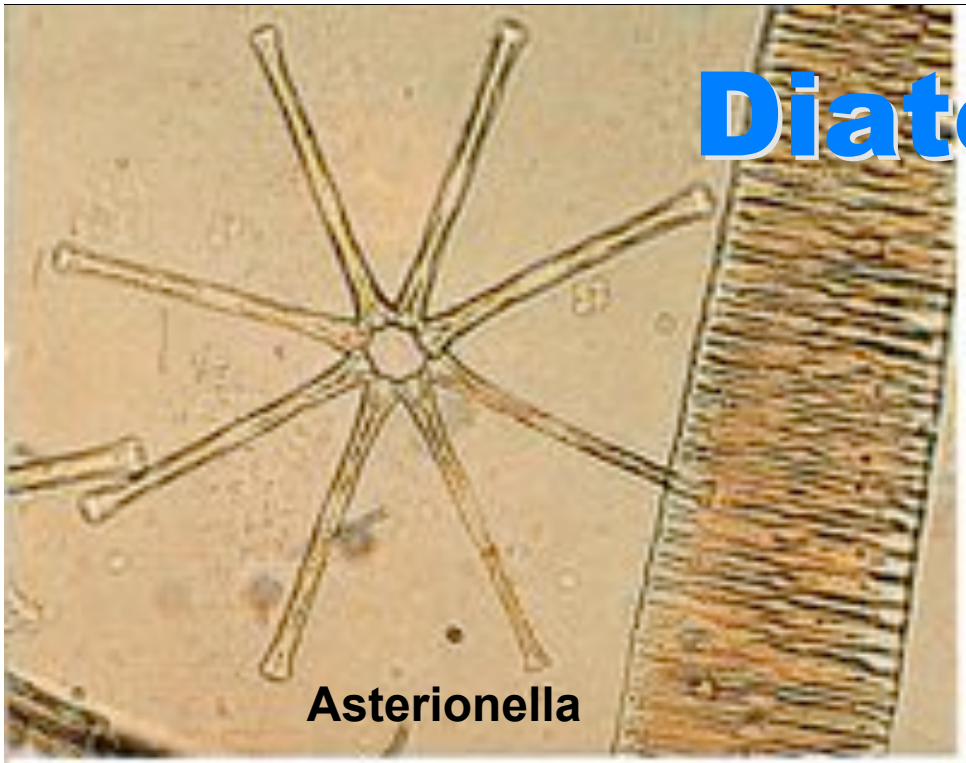
- pH > 11
- Chlorine
  - 0.5 ppm, pH 7.8, 2°C, 3 to 60 min. contact time ⊥99.99% kill
  - free chlorine 50 times more effective than chloramines
- Ultraviolet Light
- Ozone (very effective)
- Heat (60°C)

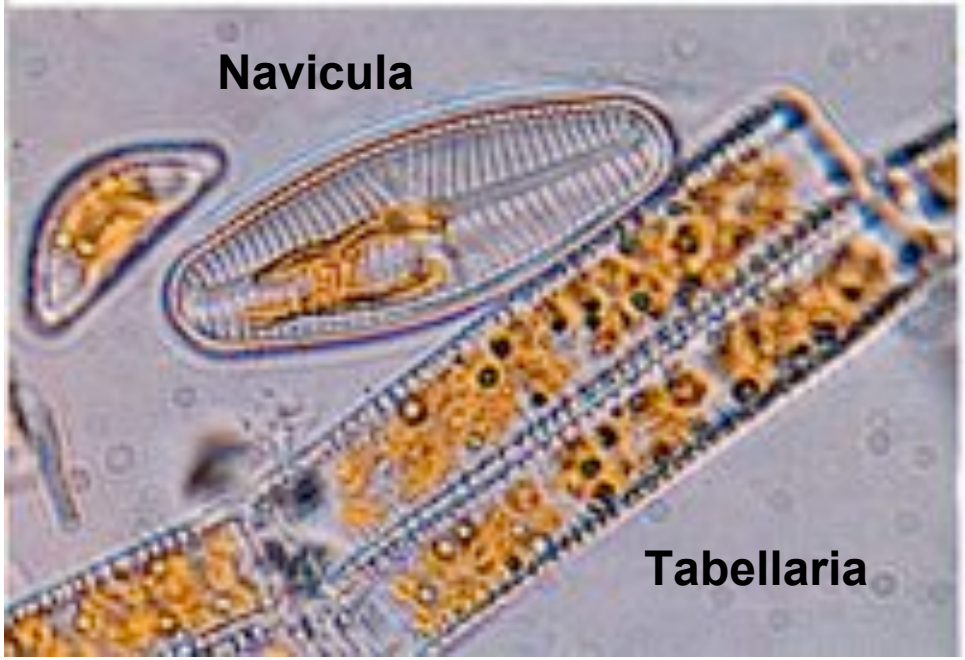
Algae

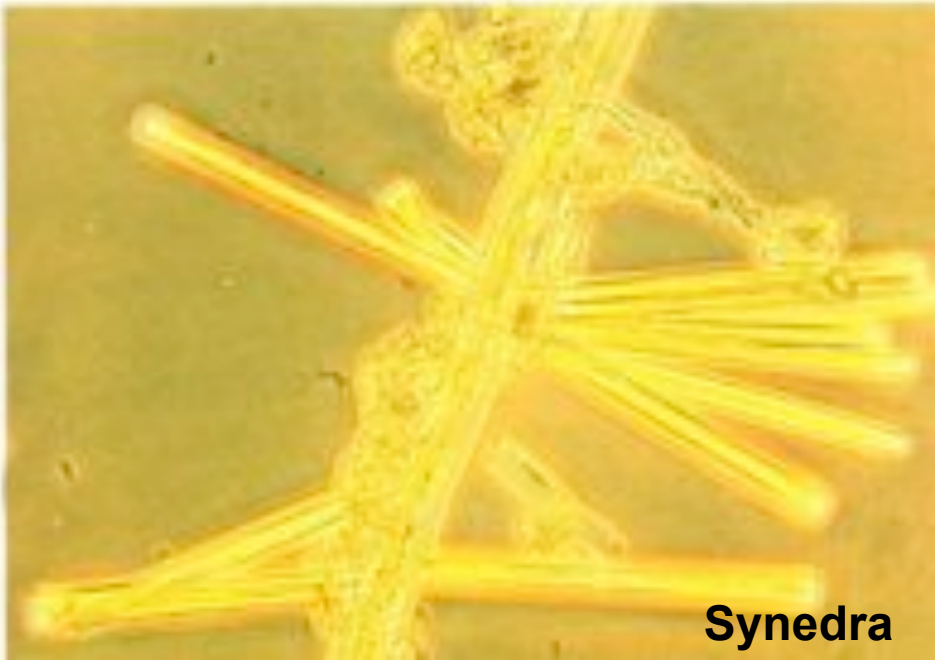
Algae Color Plate

Standard Methods

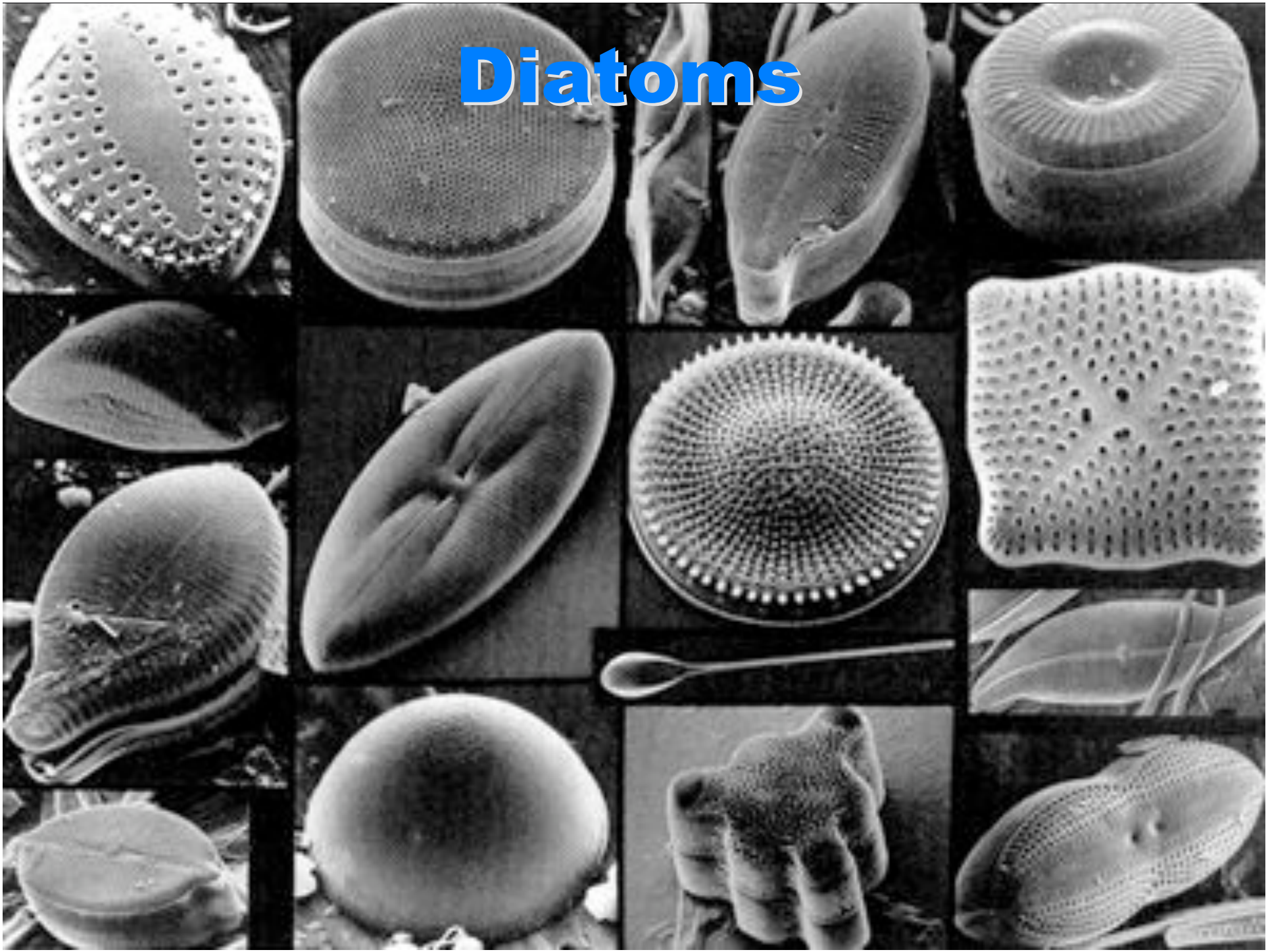
Diatoms







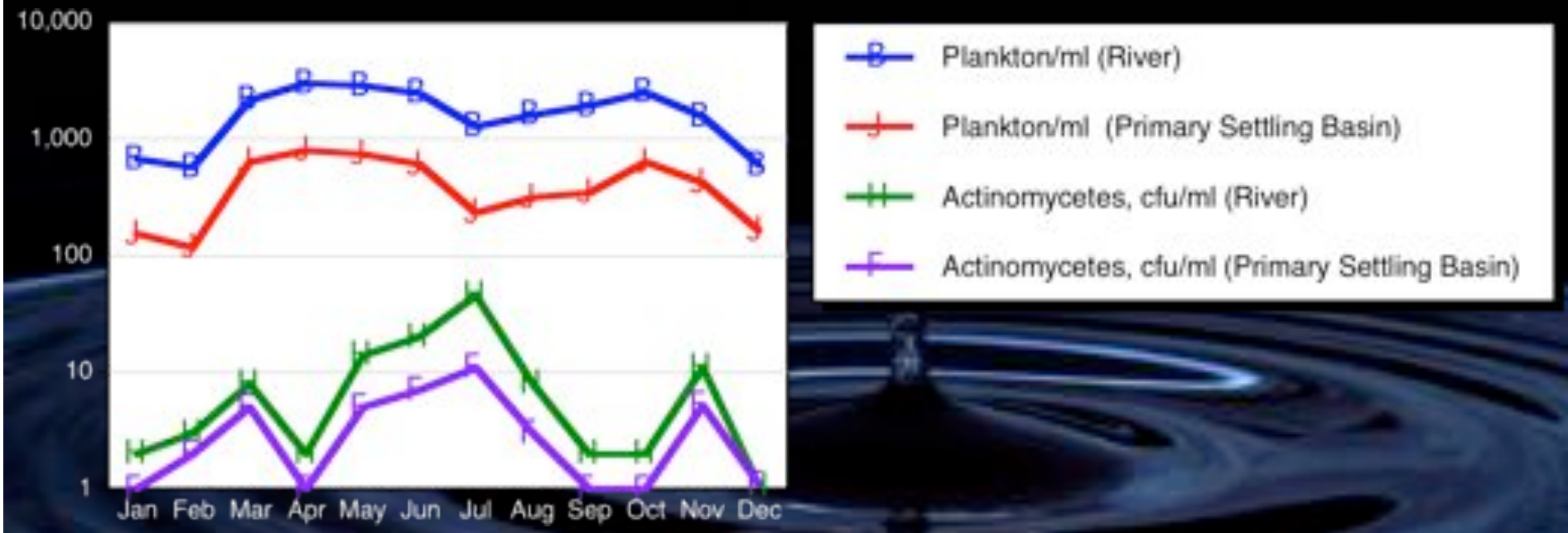
Diatoms



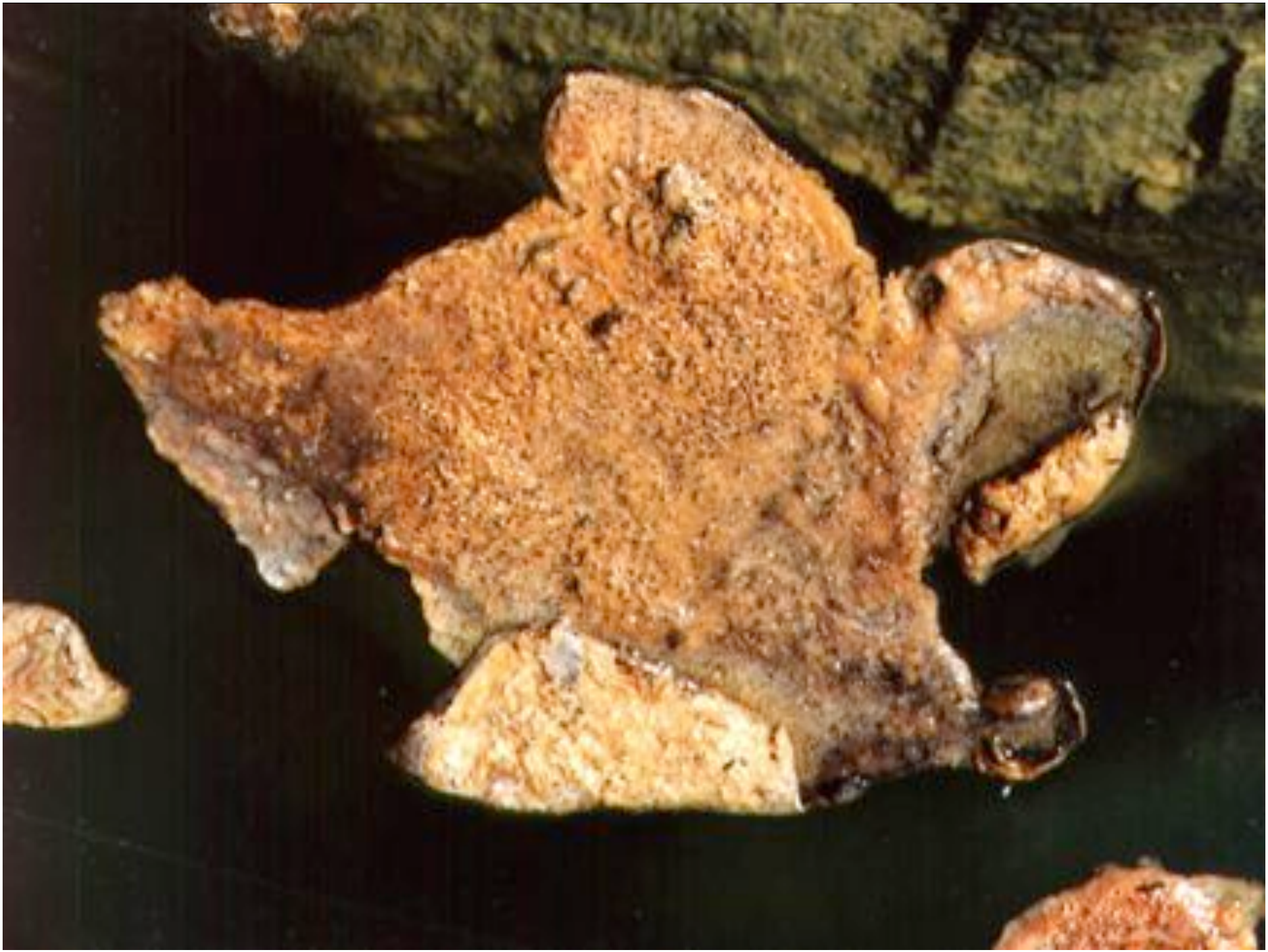
Volvox and Synura

Dinoflagellate

Plankton & Actinomycetes

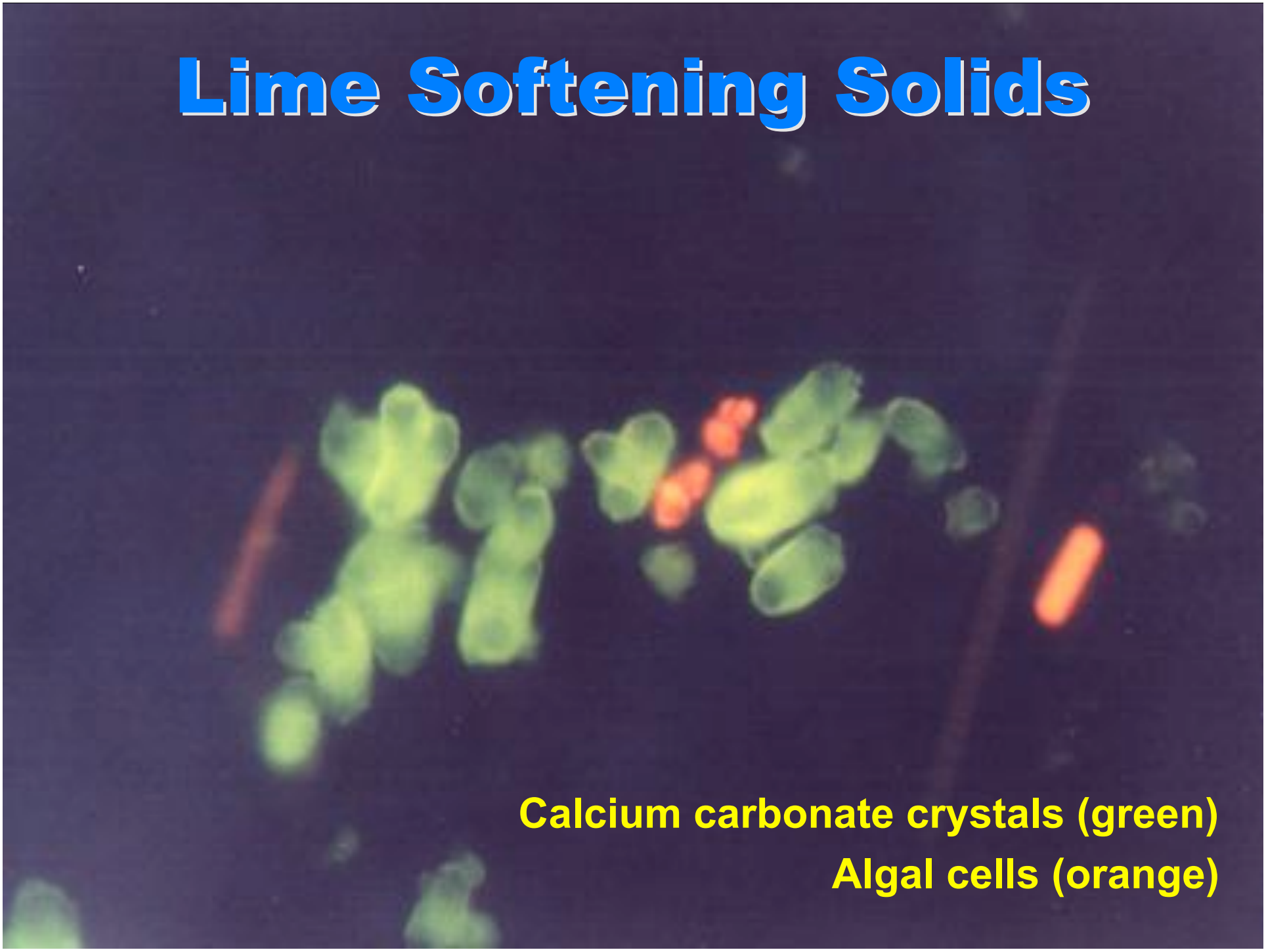


Floating Solids



Micrograph of Floating Solids

Lime Softening Solids

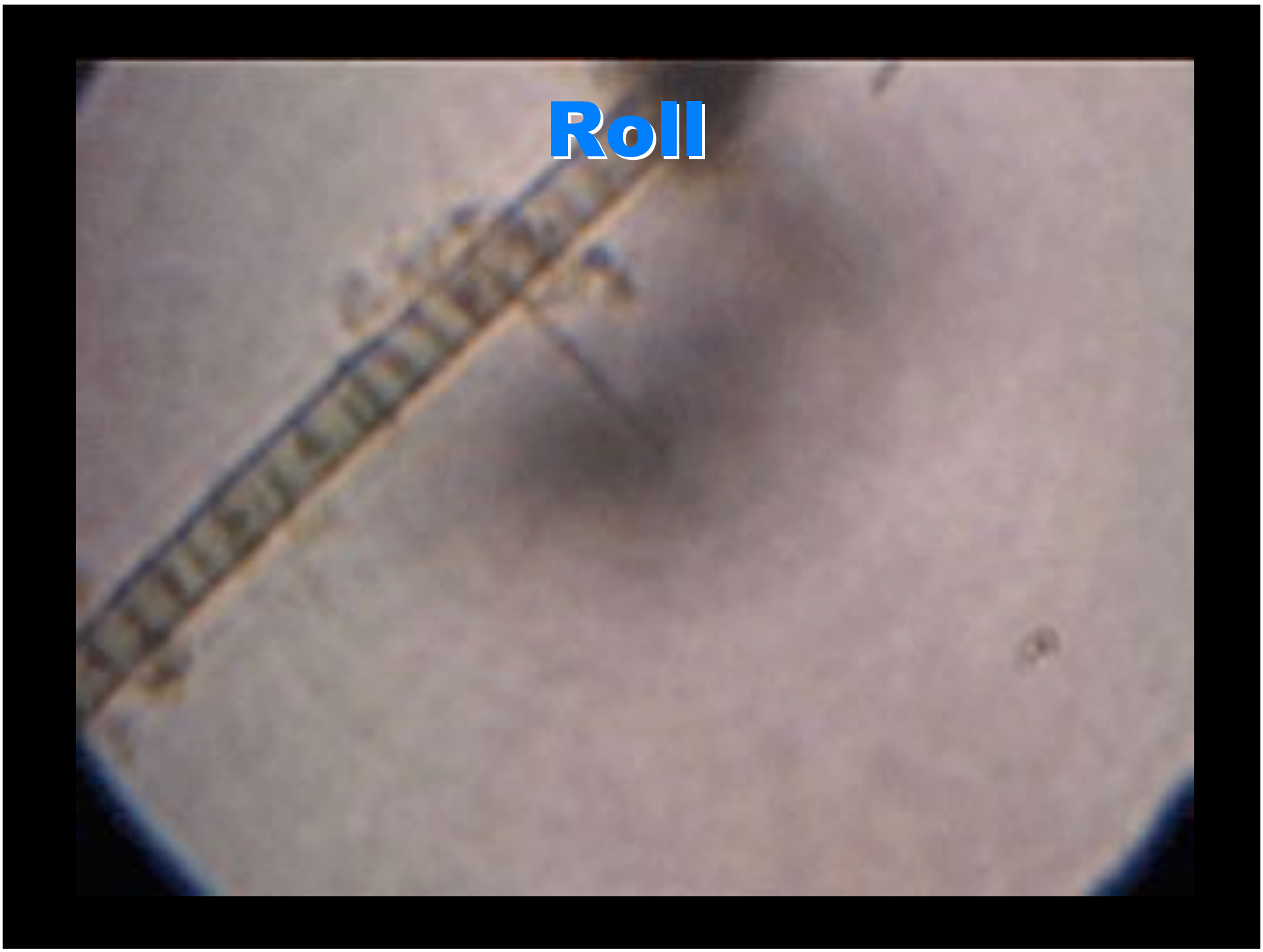


Calcium carbonate crystals (green)

Algal cells (orange)


Algae, Bacteria, $CaCO_3$

Rotation


Roll

Split

Protozoan, Algae, Bacteria

Bacterial Chains & Filaments

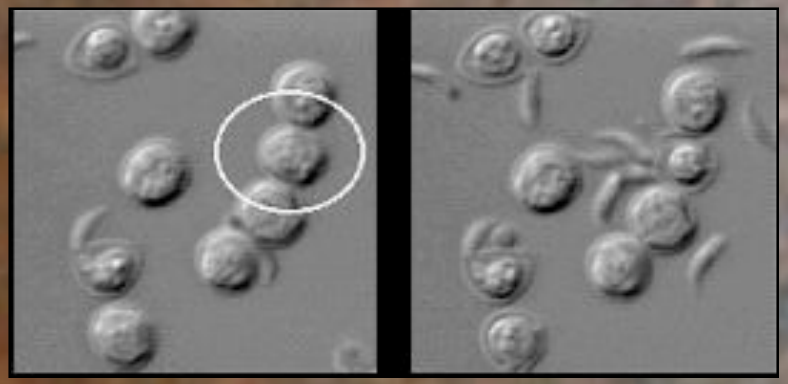
Bacterial Motion

More Chains and Filaments


Chroococcus

Nematodes in the Mat

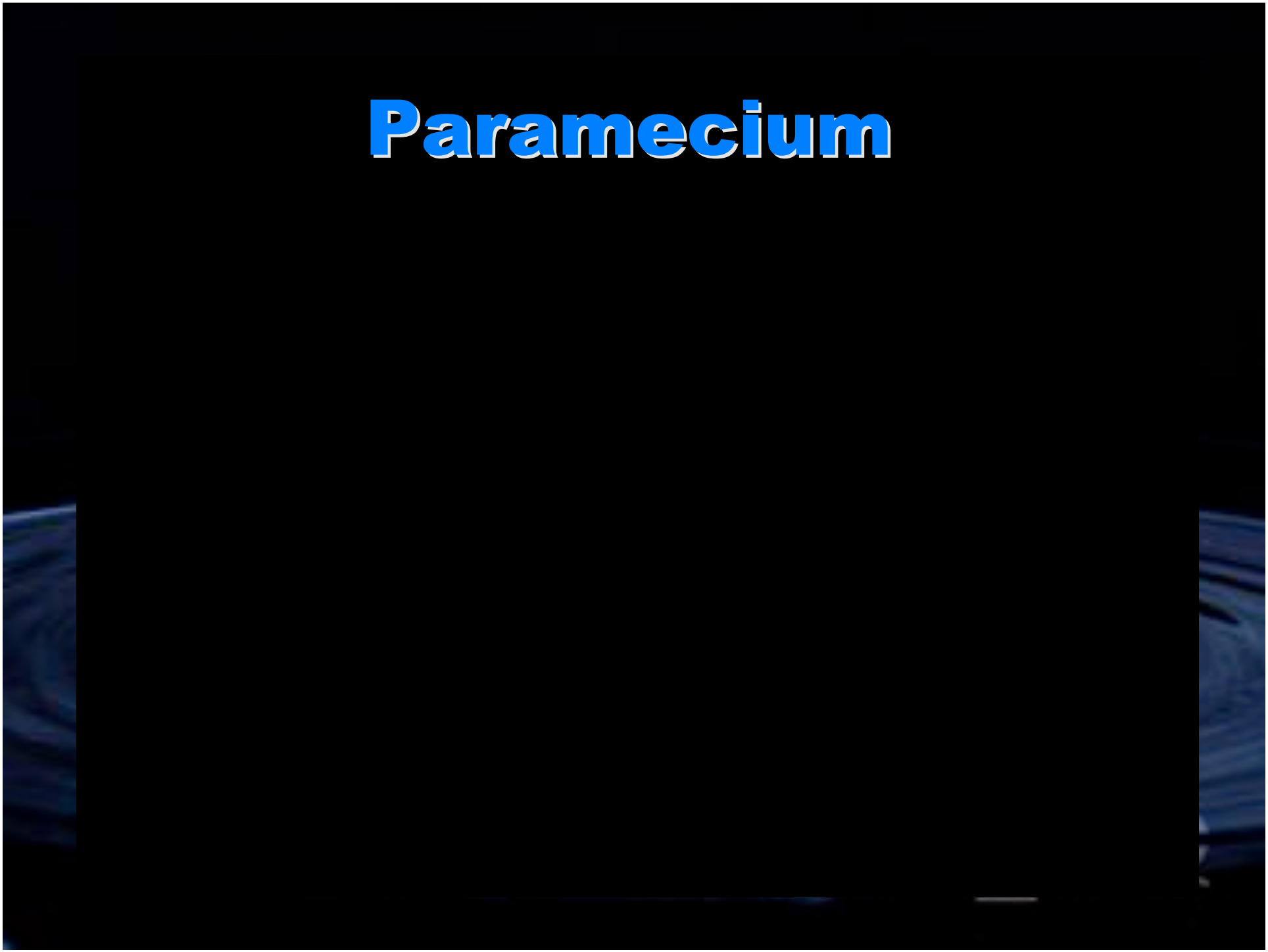
Protozoans



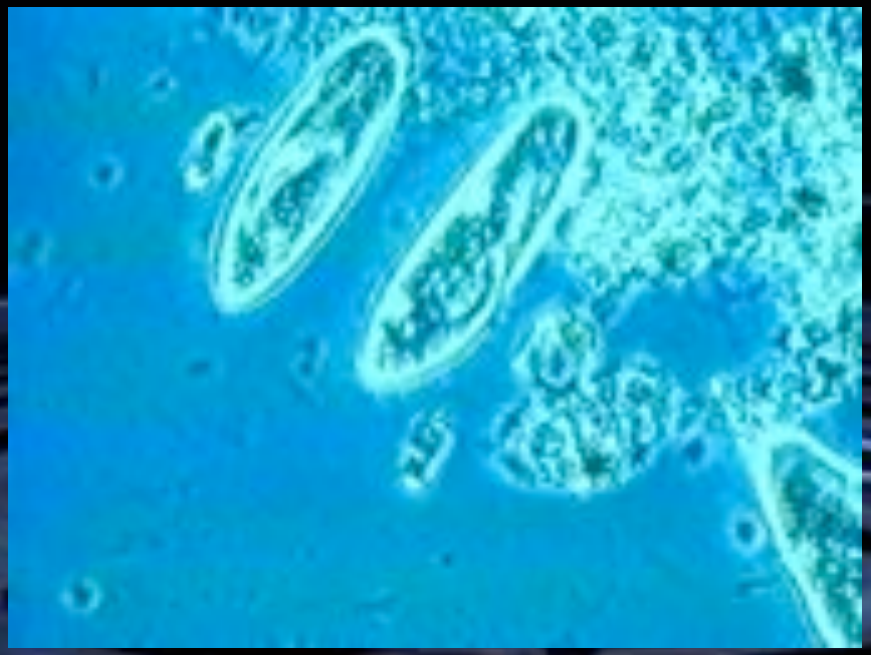
Cryptosporidium

- 40,000 species
- single-celled
- up to several mm long

Paramecium

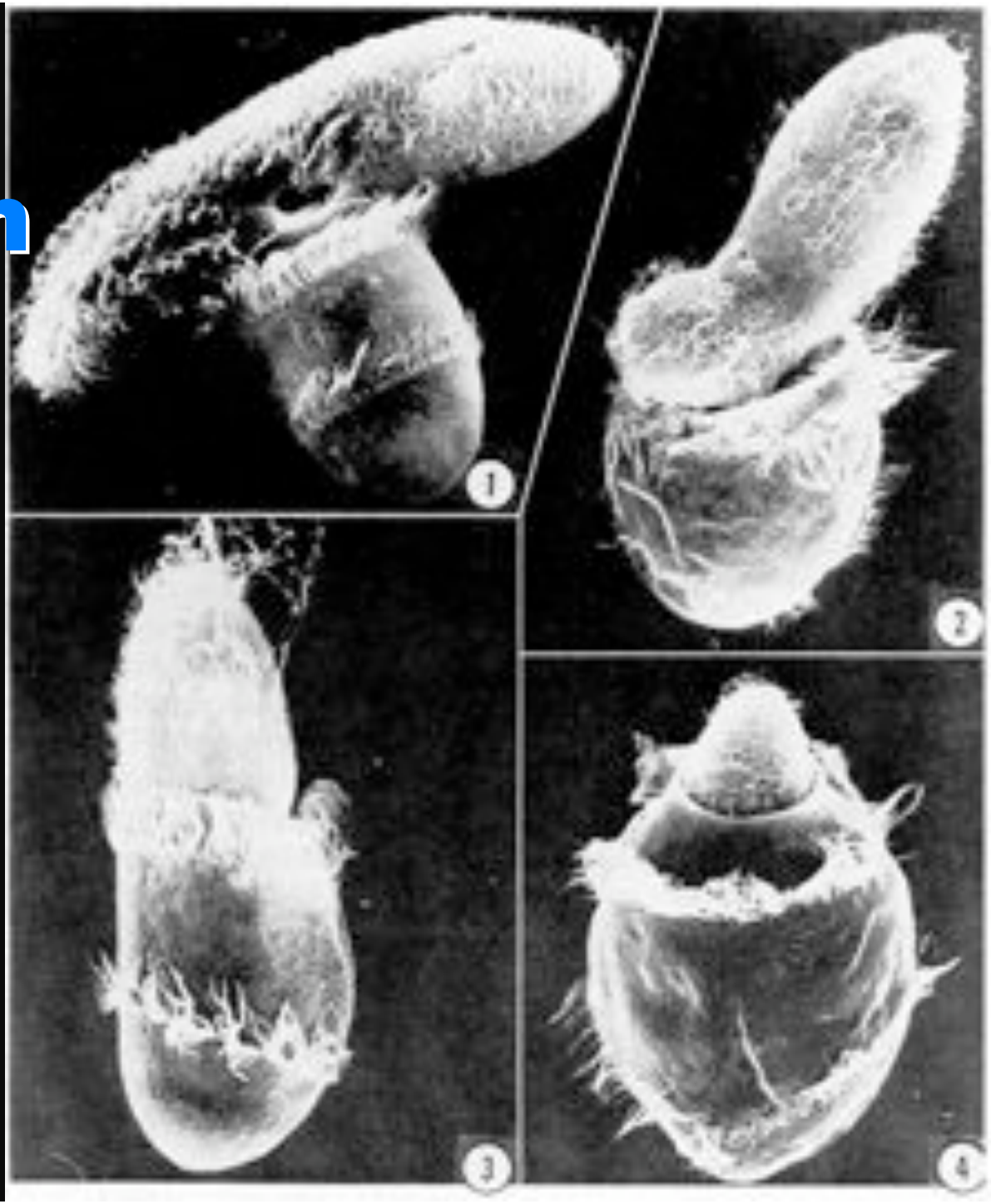


Paramecium cilia



Life in the Food Chain

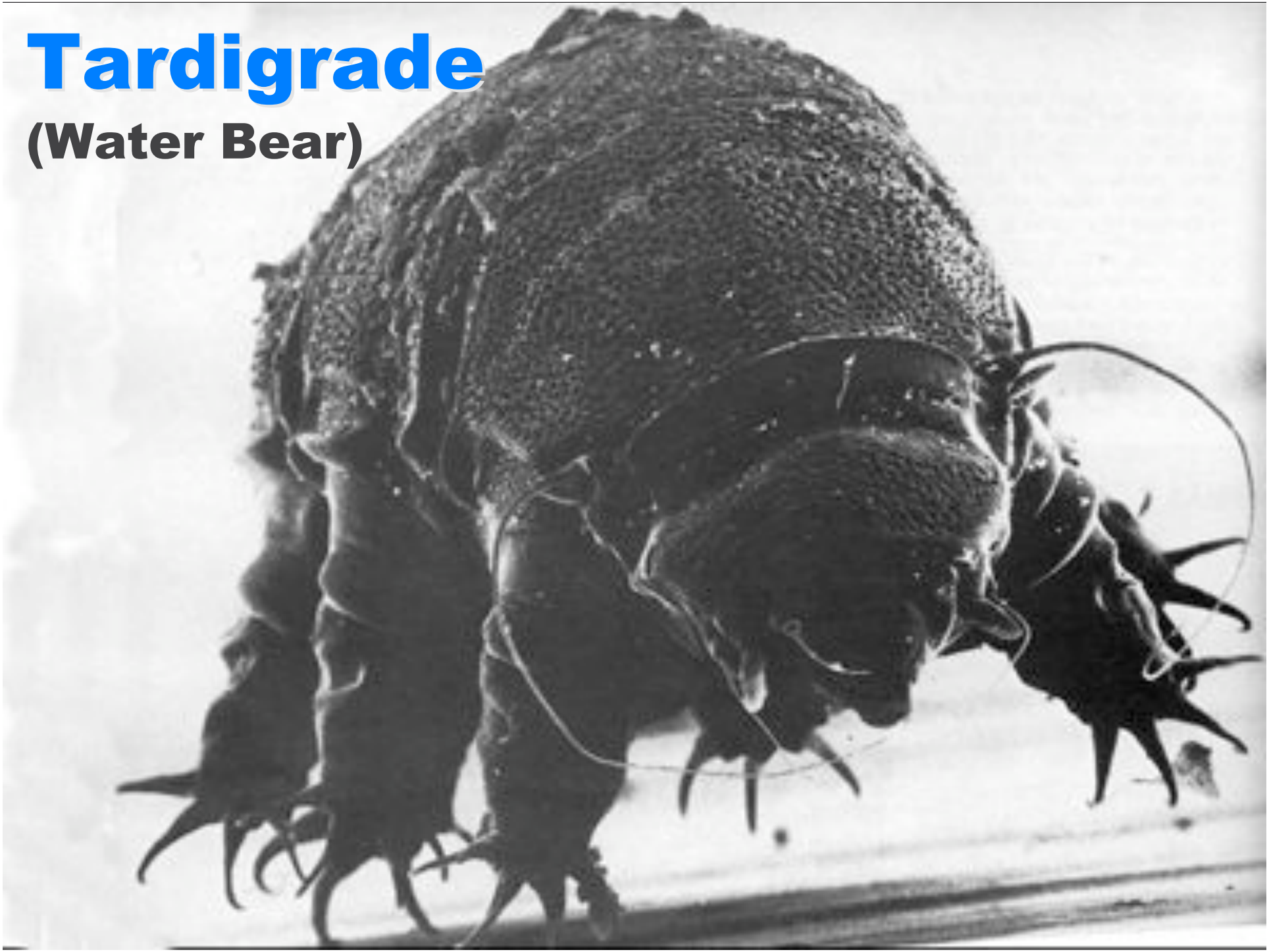
Didinium catches, eats a Paramecium



Ameba

Tardigrade

(Water Bear)



Ailments
Attributed to Protozoans

amoebic dysentary (Entamoeba histolytica)
giardiasis (Giardia lamblia)
giant roundworm (Ascaris lumbricoides)
cryptosporidiosis (Cryptosporidium)

Lines of Defense

- Source Water Protection
- Physical Removal
- Kill / Inactivation
- Disinfectant Residual
- Maintain Integrity of Distribution and Storage