

A microscopic view of a wastewater floc, showing a complex network of fine, interconnected fibers and small, dark, irregular particles. The overall appearance is that of a dense, tangled mesh. The background is a light, slightly grainy grey.

Bugs and Critters: Life in the Floc

Operator Training Committee of Ohio

Wastewater Operator's Workshop

Deer Creek State Park

May 14, 2014

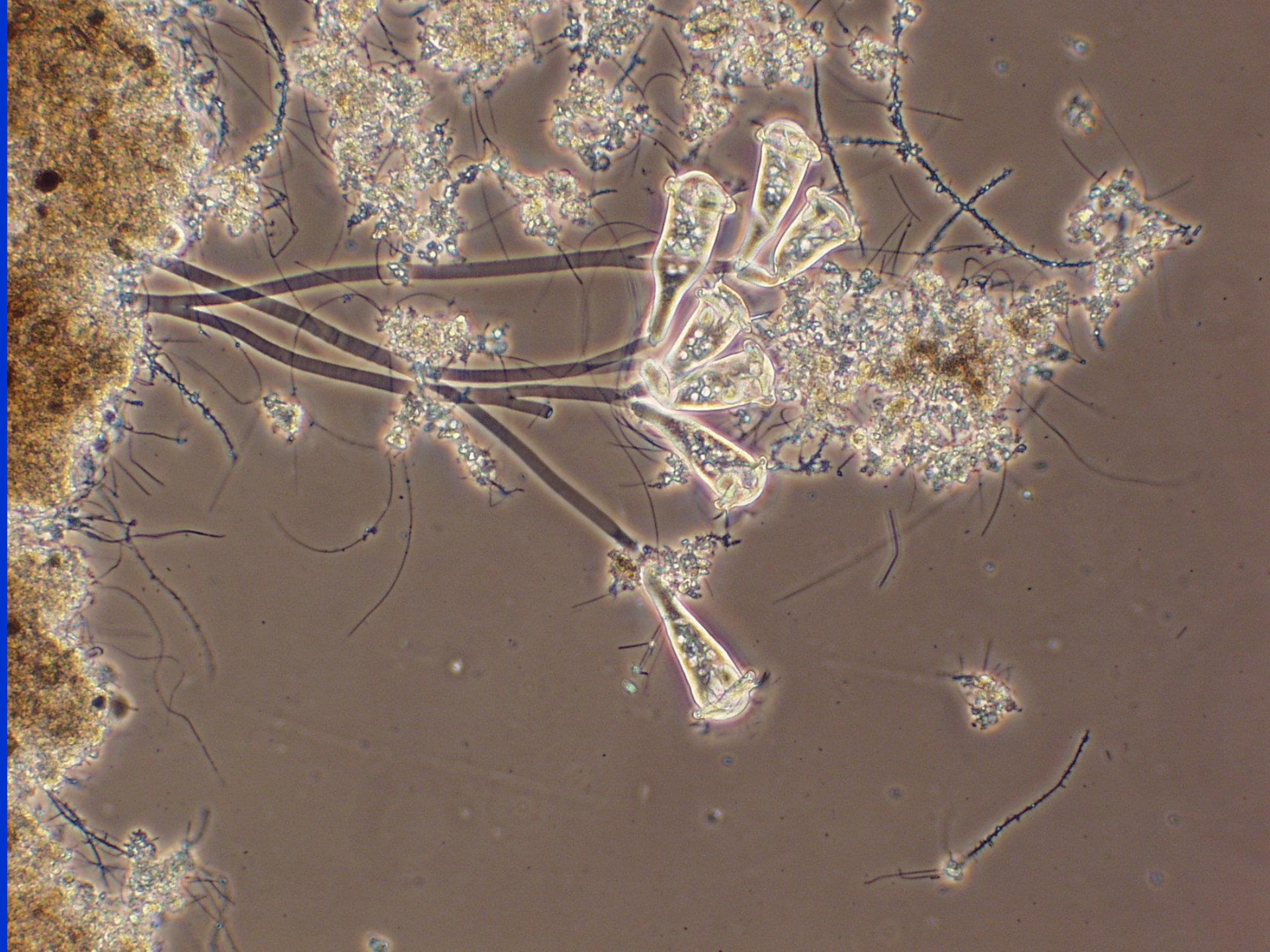
Ohio EPA Compliance Assistance Unit

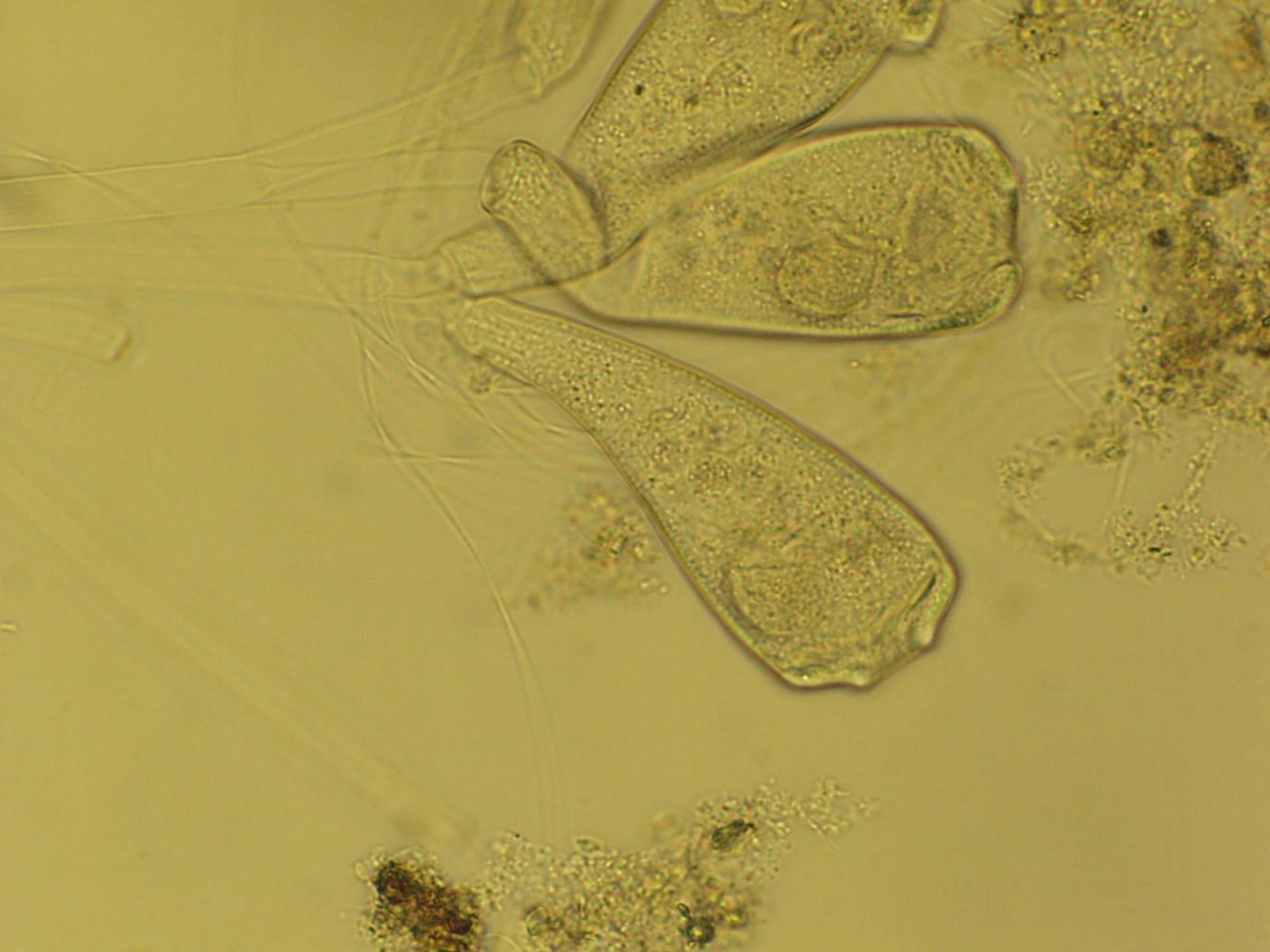
A microscopic image showing a complex network of thin, dark, branching structures, possibly fungal hyphae or plant roots, against a light gray background. The structures are dense and interconnected, with some larger, more rounded clusters. The overall appearance is that of a highly branched, filamentous organism.

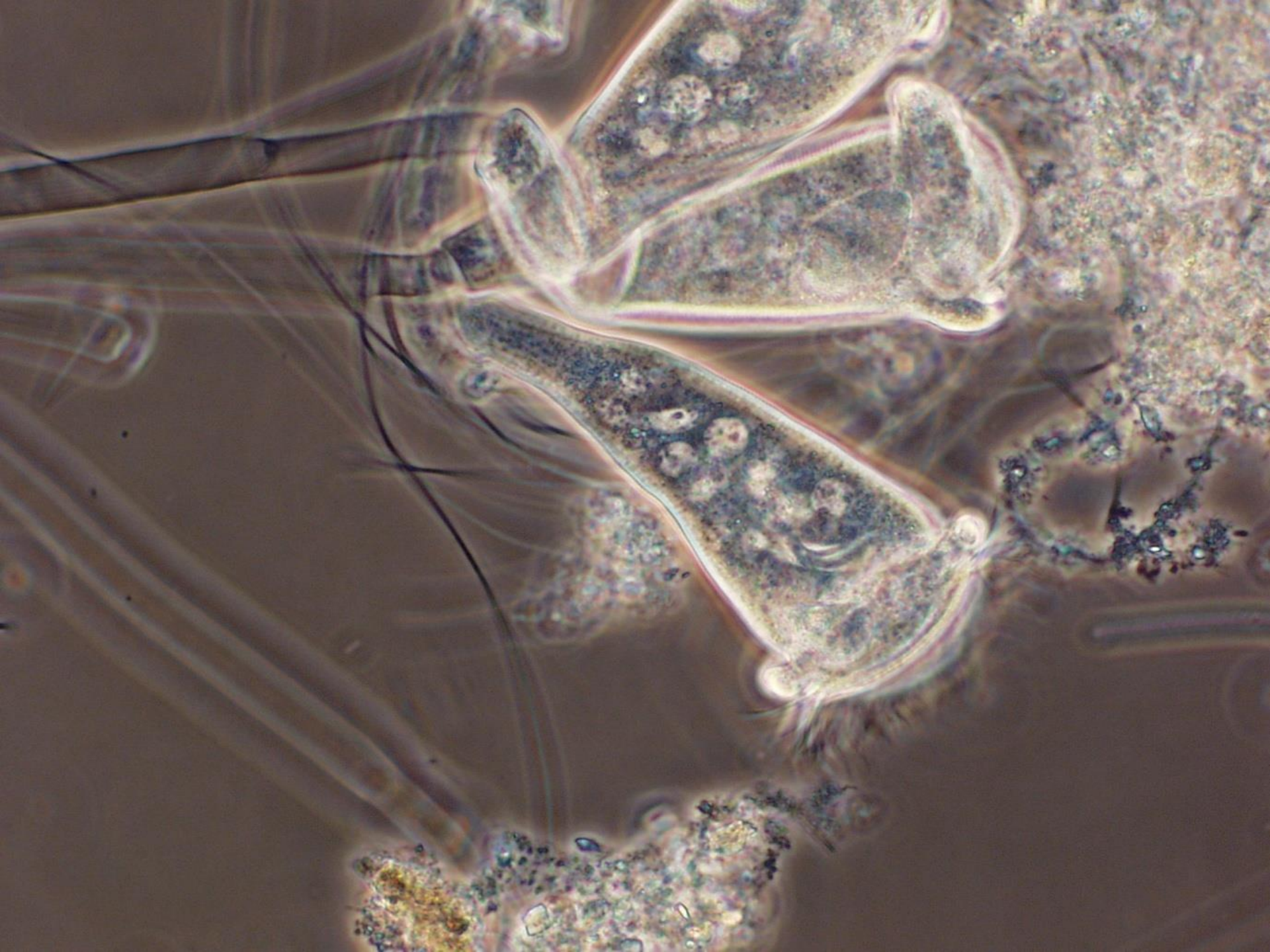
**Why Does My Microscopic
Work Better than Yours?**





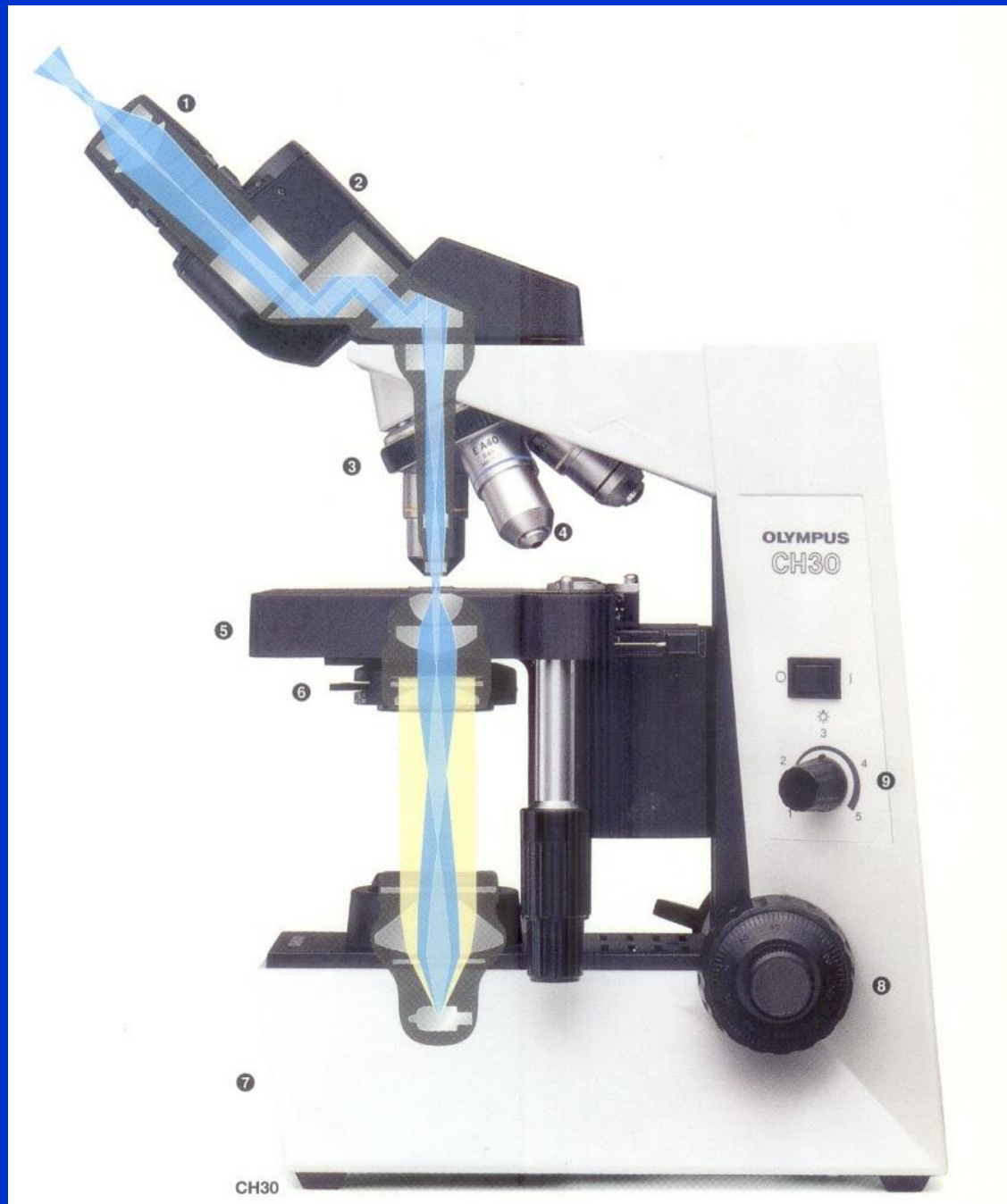






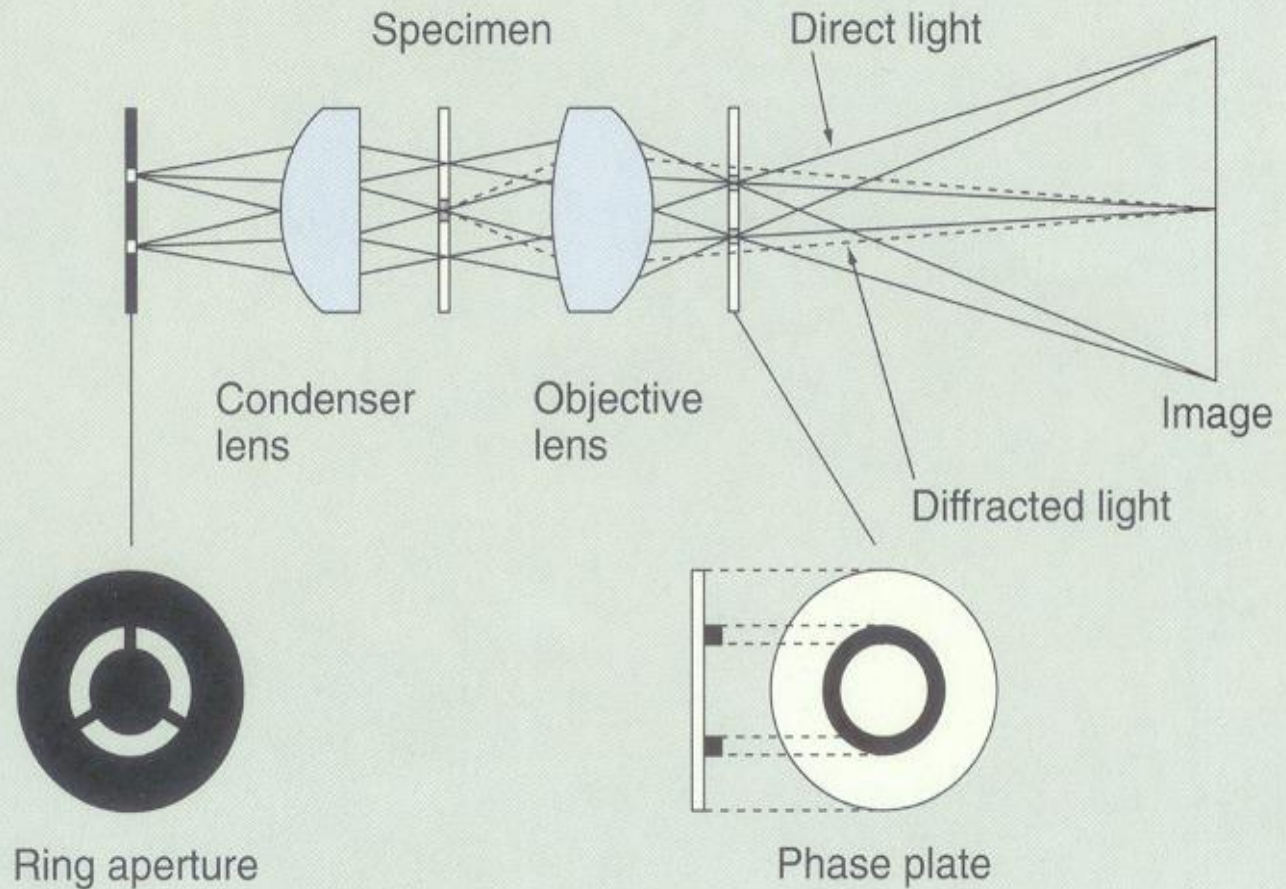






CH30

Fig. 1. Phase Contrast Microscopy



A microscopic image showing a complex network of thin, dark, branching structures, possibly fungal hyphae or plant roots, against a light gray background. The structures are interconnected and form a dense, web-like pattern. The text "Why Perform a Microscopic Analysis?" is overlaid in the center in a bold, black, sans-serif font.

Why Perform a Microscopic Analysis?

Essential Resources



Process Control of
Activated Sludge Plants by
Microscopic Investigation


Dick H Eikelboom

asis

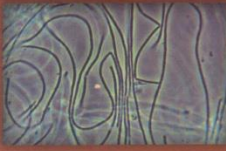
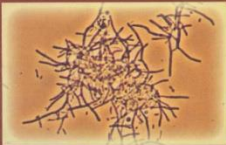
stowa

IWA
Publishing

3rd Edition

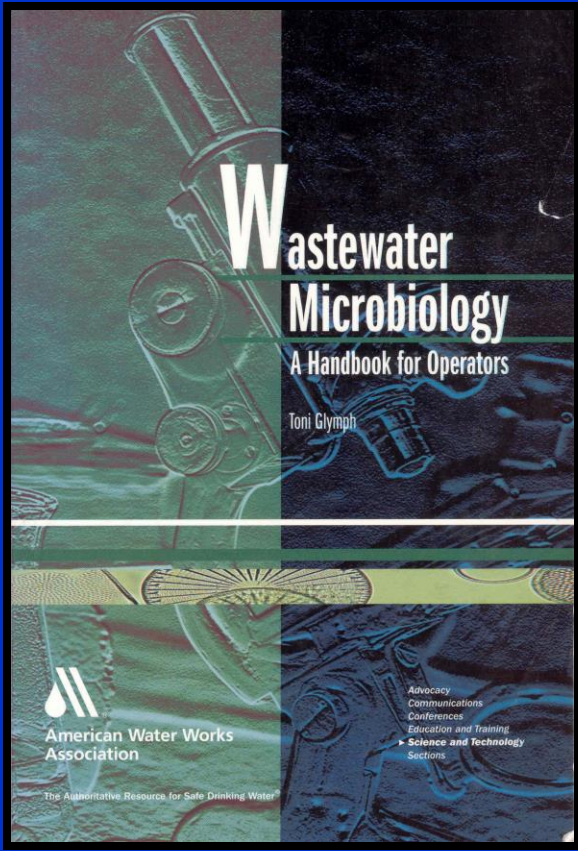


MANUAL
on the
**CAUSES and
CONTROL** of
**ACTIVATED SLUDGE
BULKING, FOAMING,
and OTHER SOLIDS
SEPARATION
PROBLEMS**



David Jenkins
Michael G. Richard
Glen T. Daigger

LEWIS PUBLISHERS



Wastewater
Microbiology

A Handbook for Operators

Toni Glymph

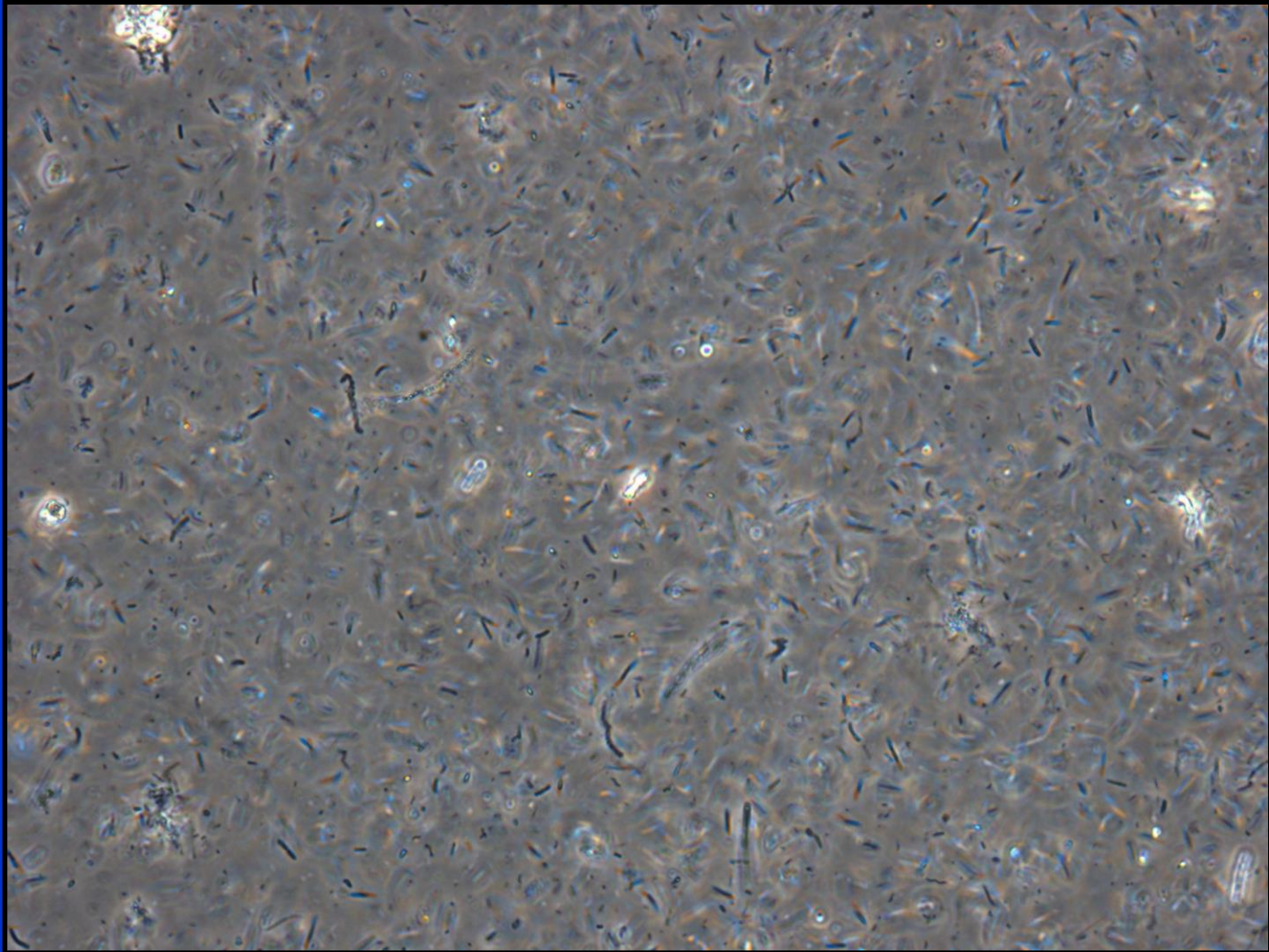


American Water Works
Association

The Authoritative Resource for Safe Drinking Water

Advocacy
Communications
Conferences
Education and Training
Science and Technology
Sections

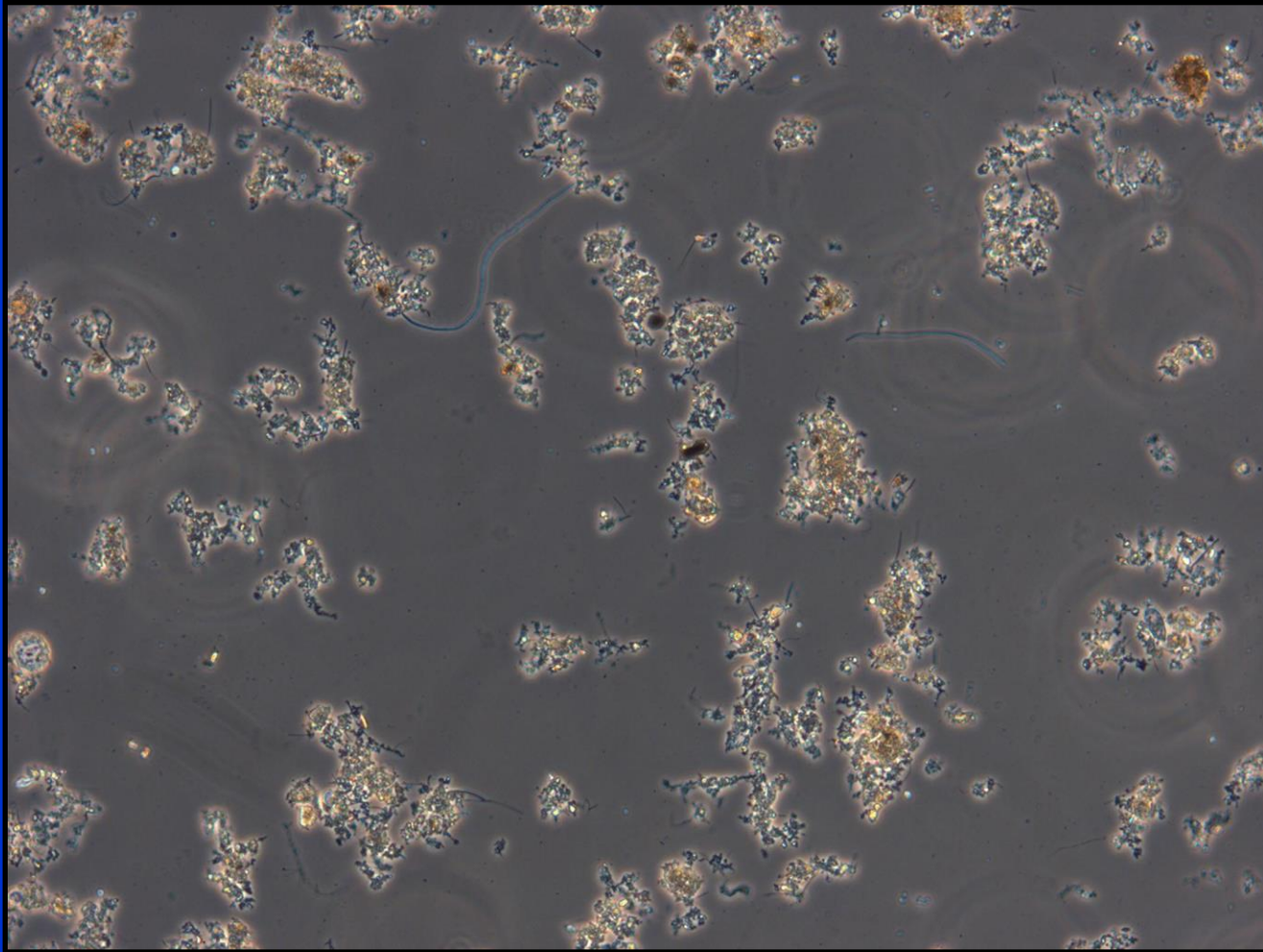
Dispersed Bacteria



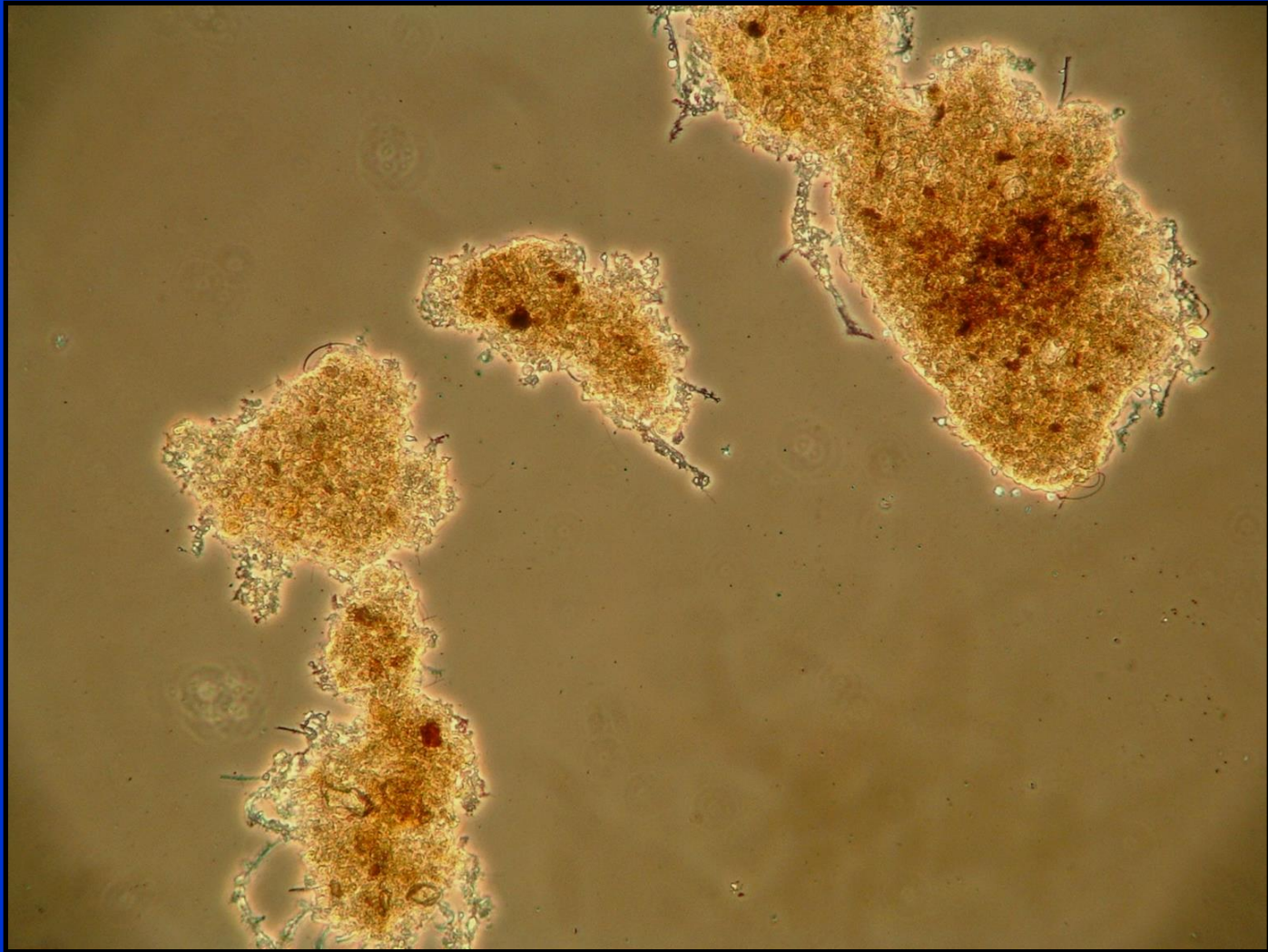
Beginning of Flocculation



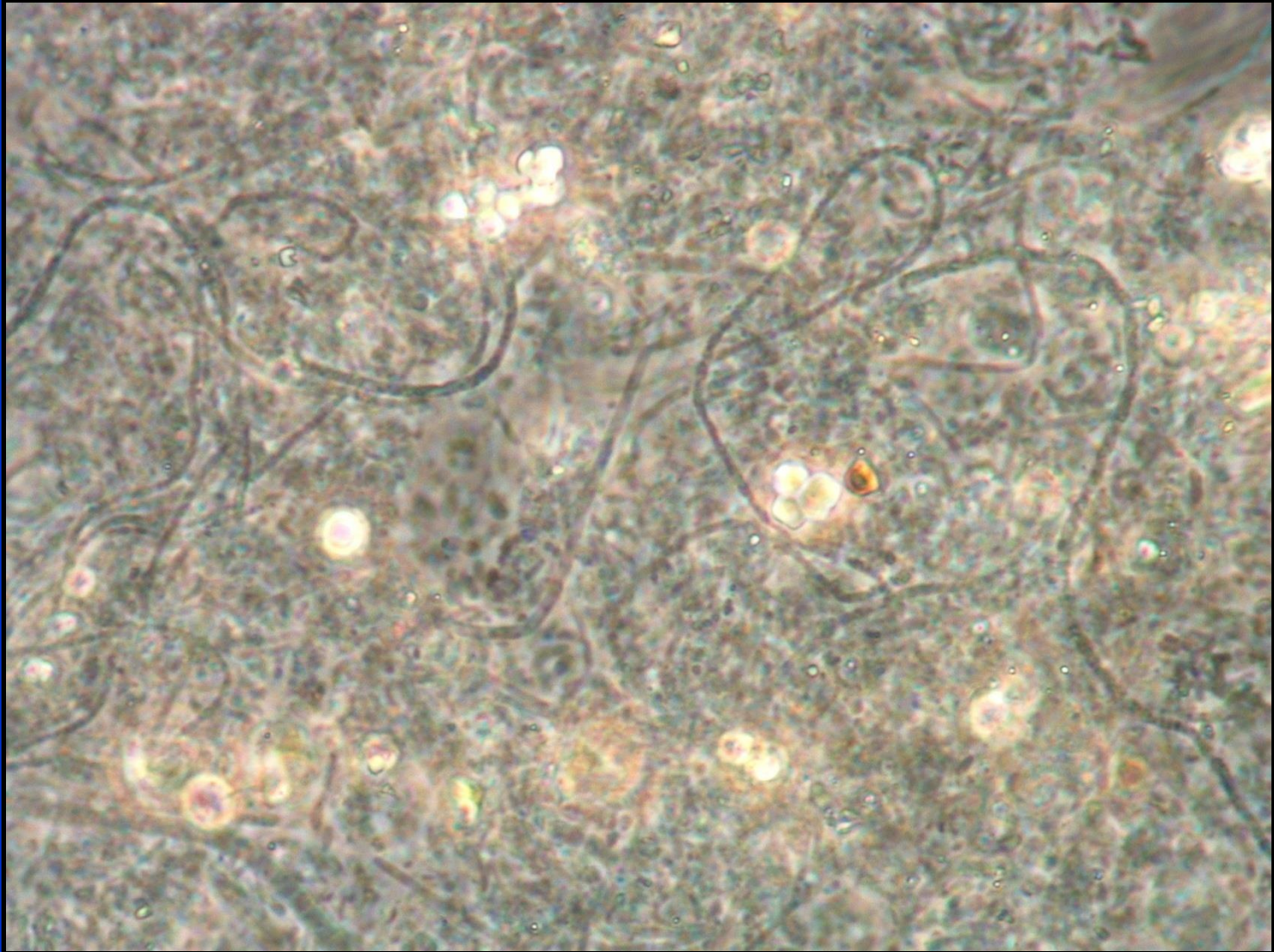
Small Flocs



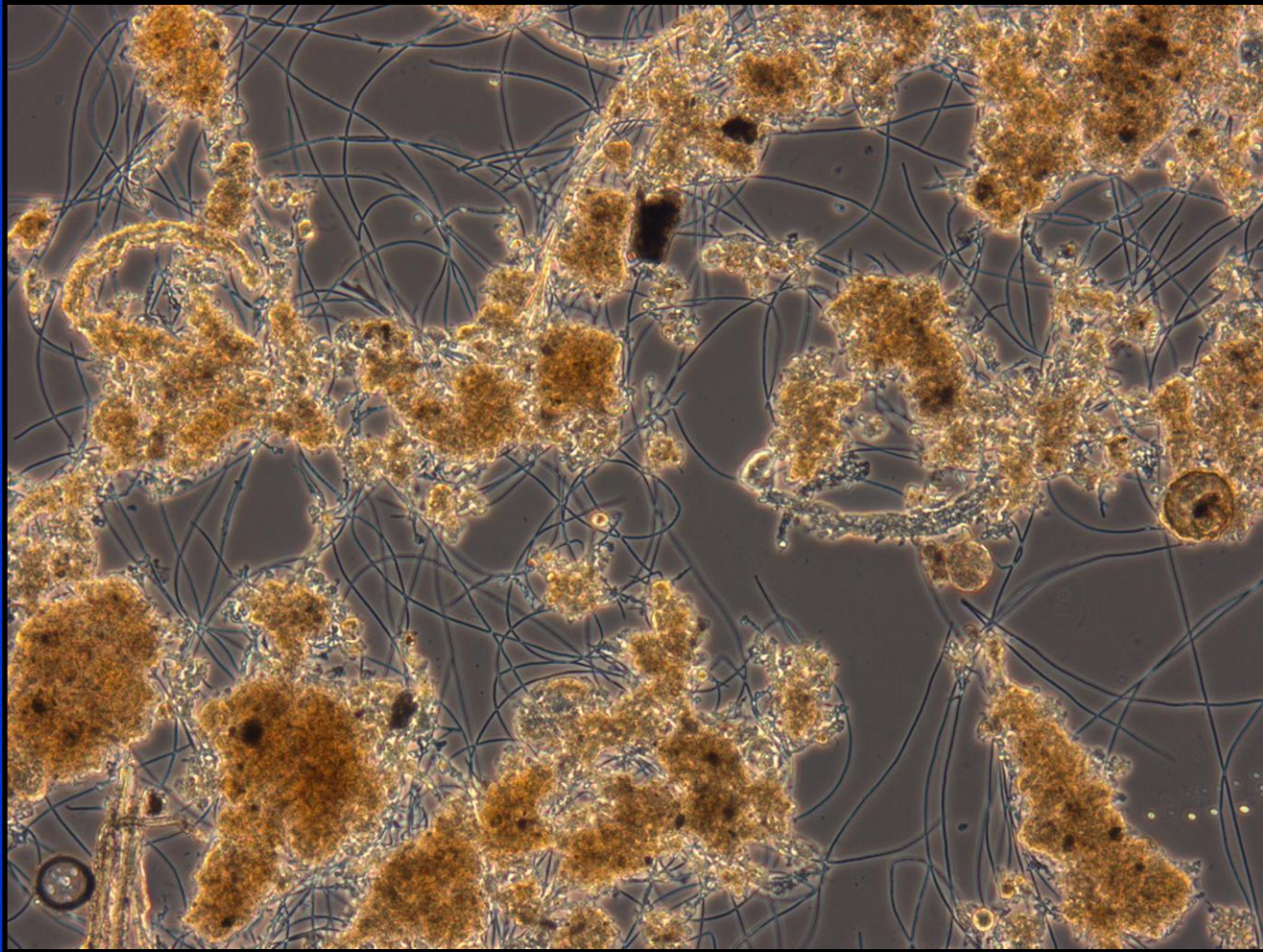
Dense, Compact Floccs



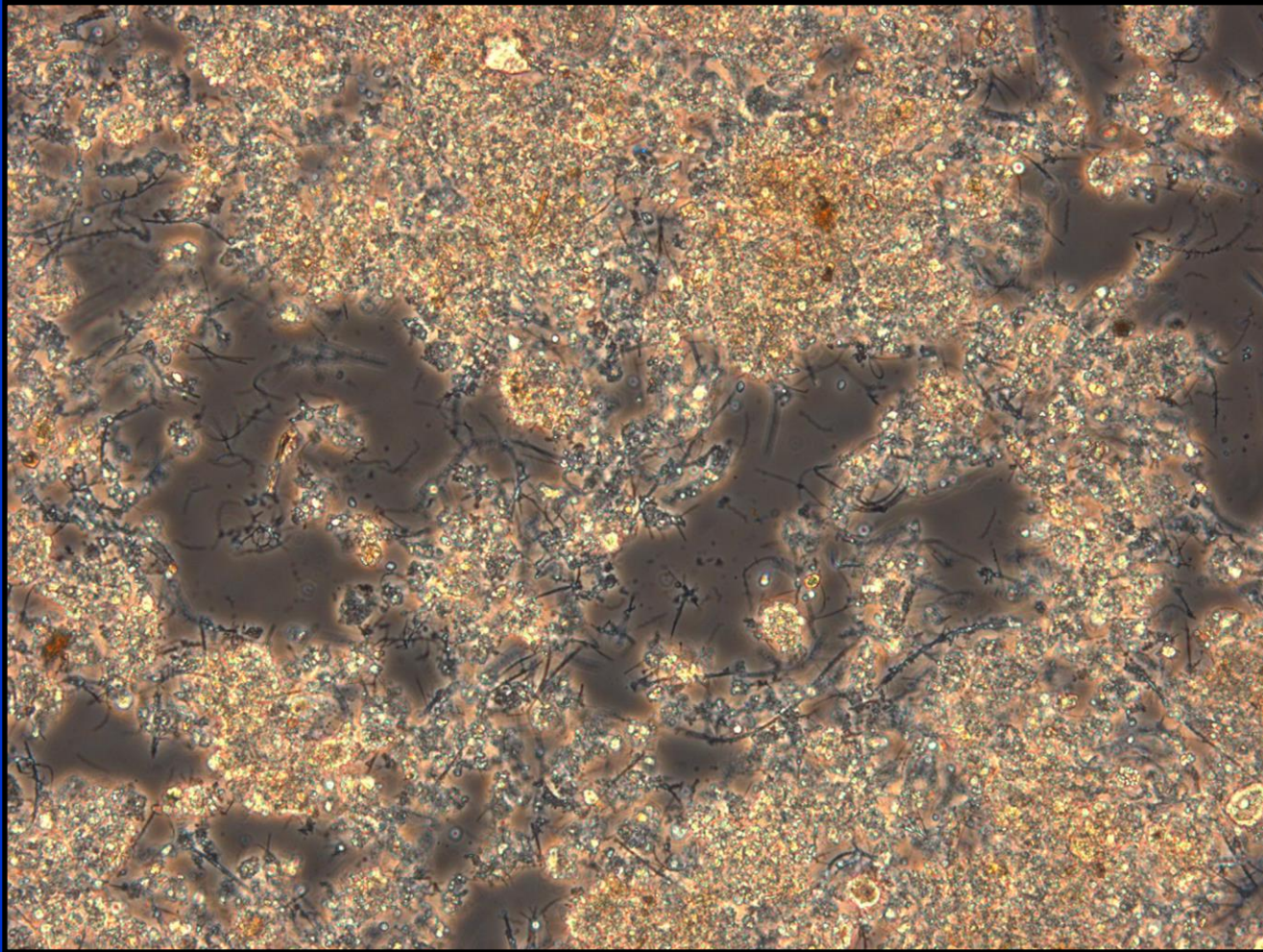
Internal Filaments Low Density Floccs



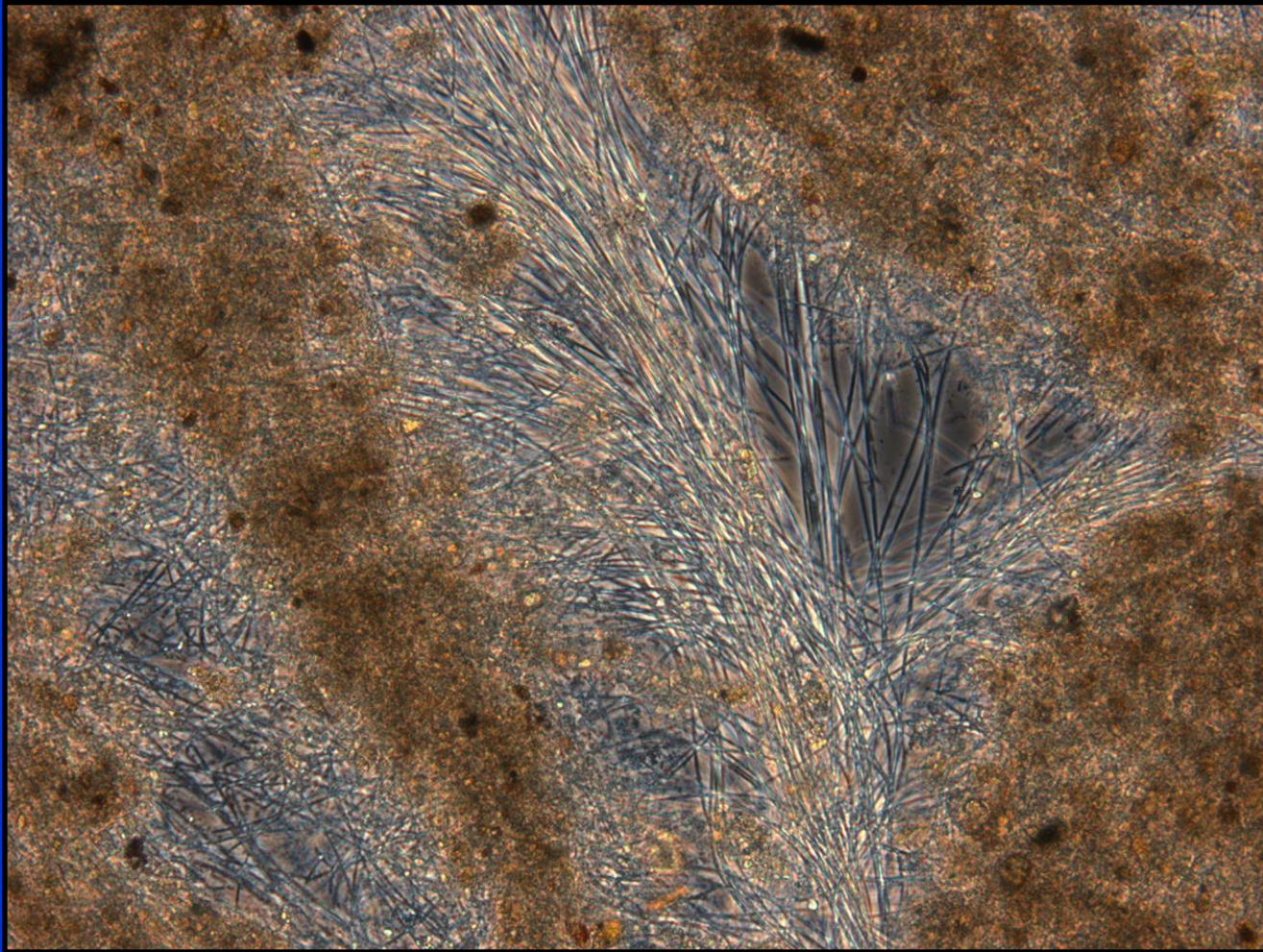
Extending Filaments Interfloc Bridging




Too Much Mass



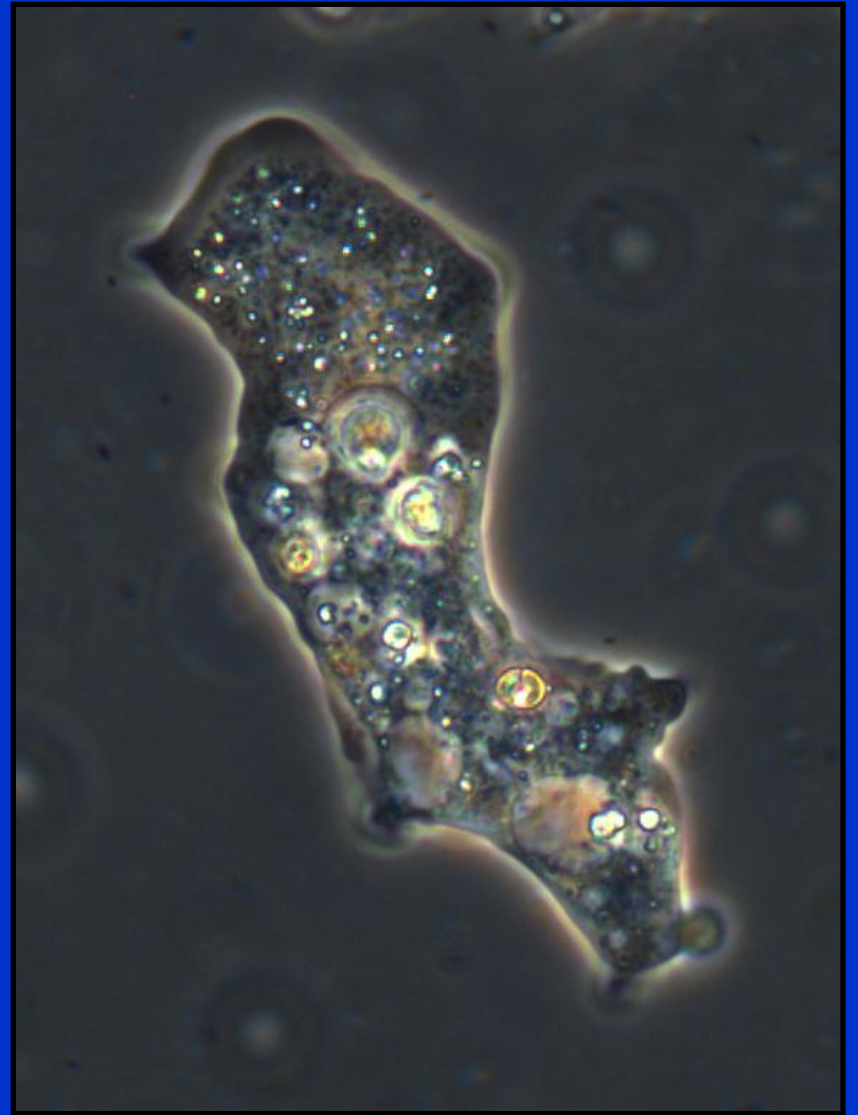
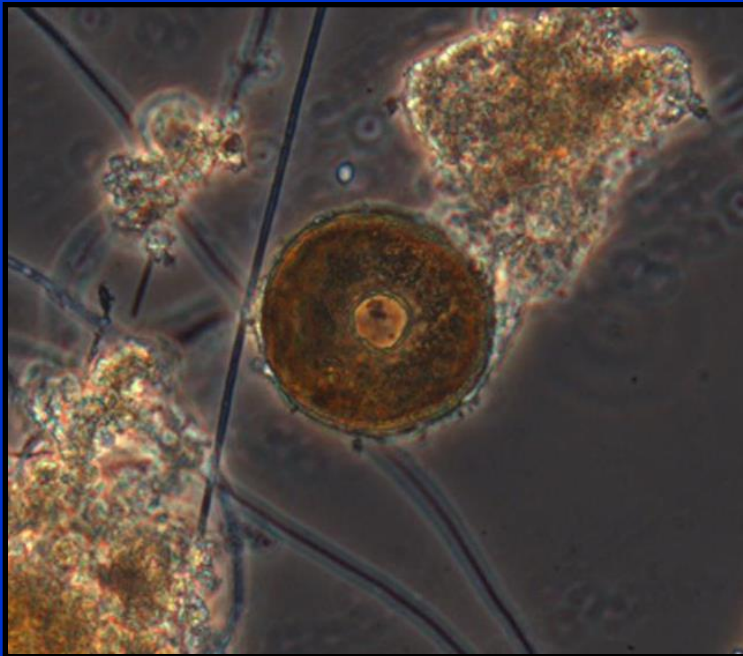
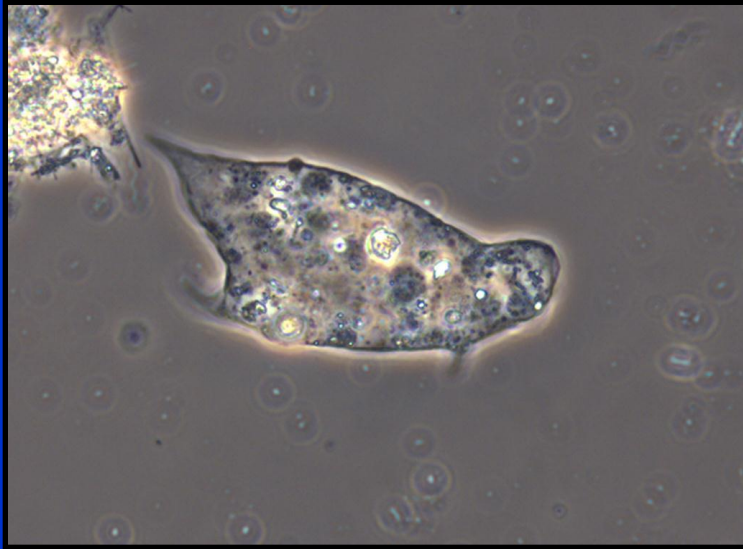
Too Much Filament



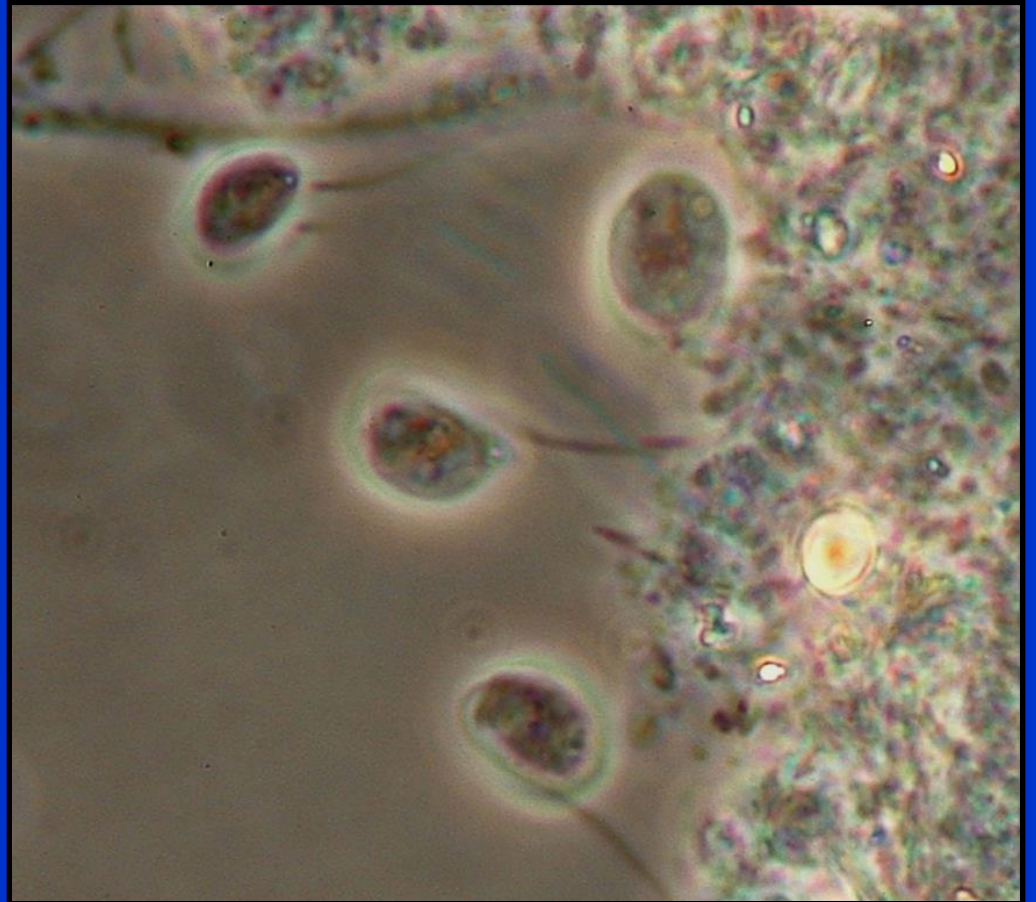
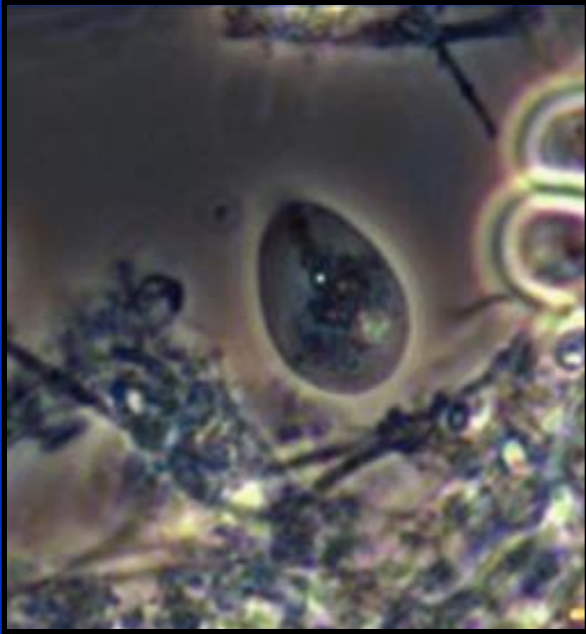
A microscopic image showing a complex network of thin, dark, branching fibers or filaments. The fibers are interconnected, forming a dense, web-like structure. There are several bright, circular spots scattered throughout the field, which could be individual cells or specific organelles. The overall appearance is that of a biological specimen, possibly a tissue section or a culture of cells, viewed under a microscope. The background is a light, slightly grainy grey.

The Protozoa and Metazoa

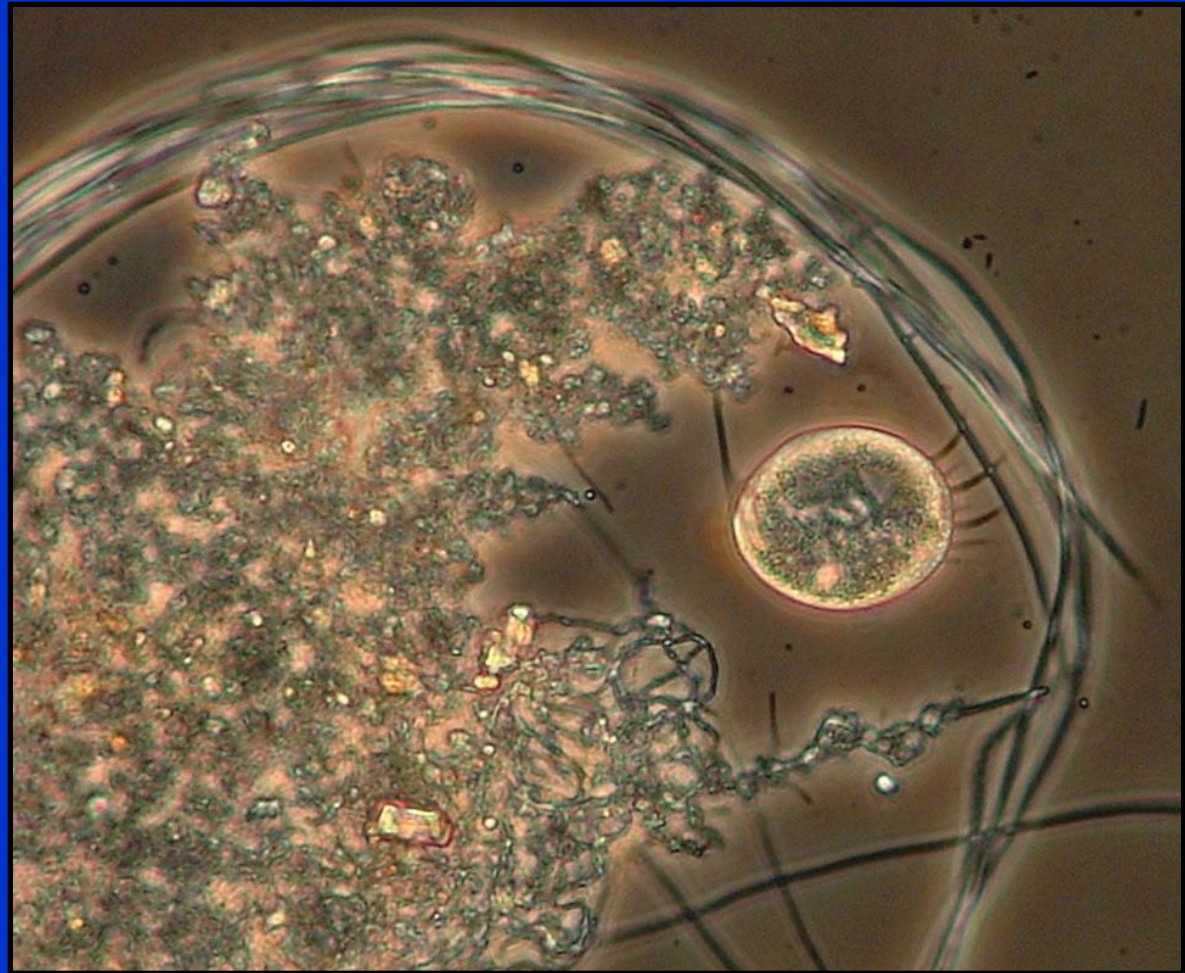
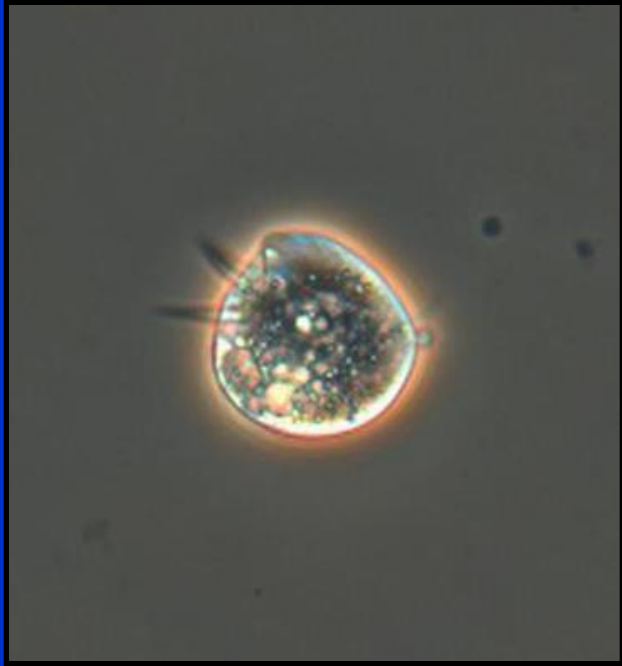
Amoeba



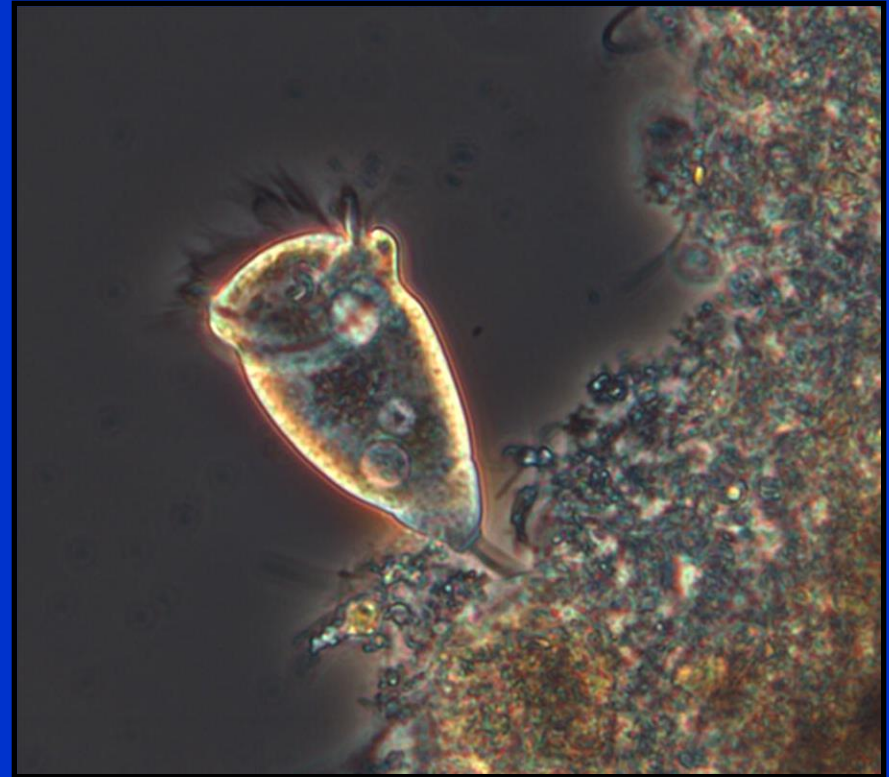
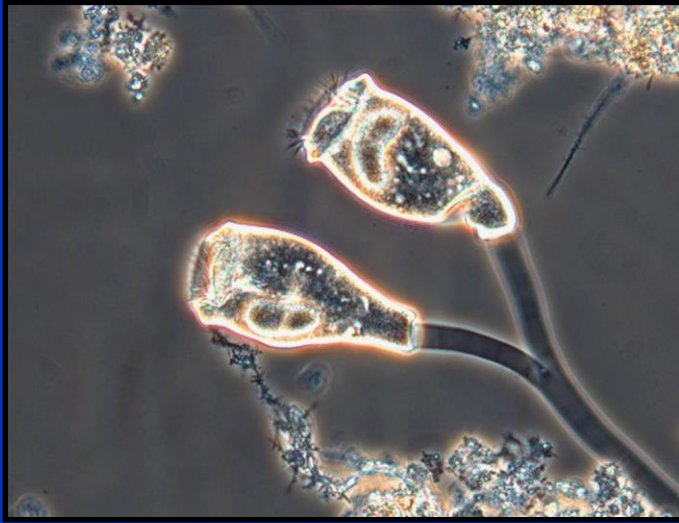
Flagellates



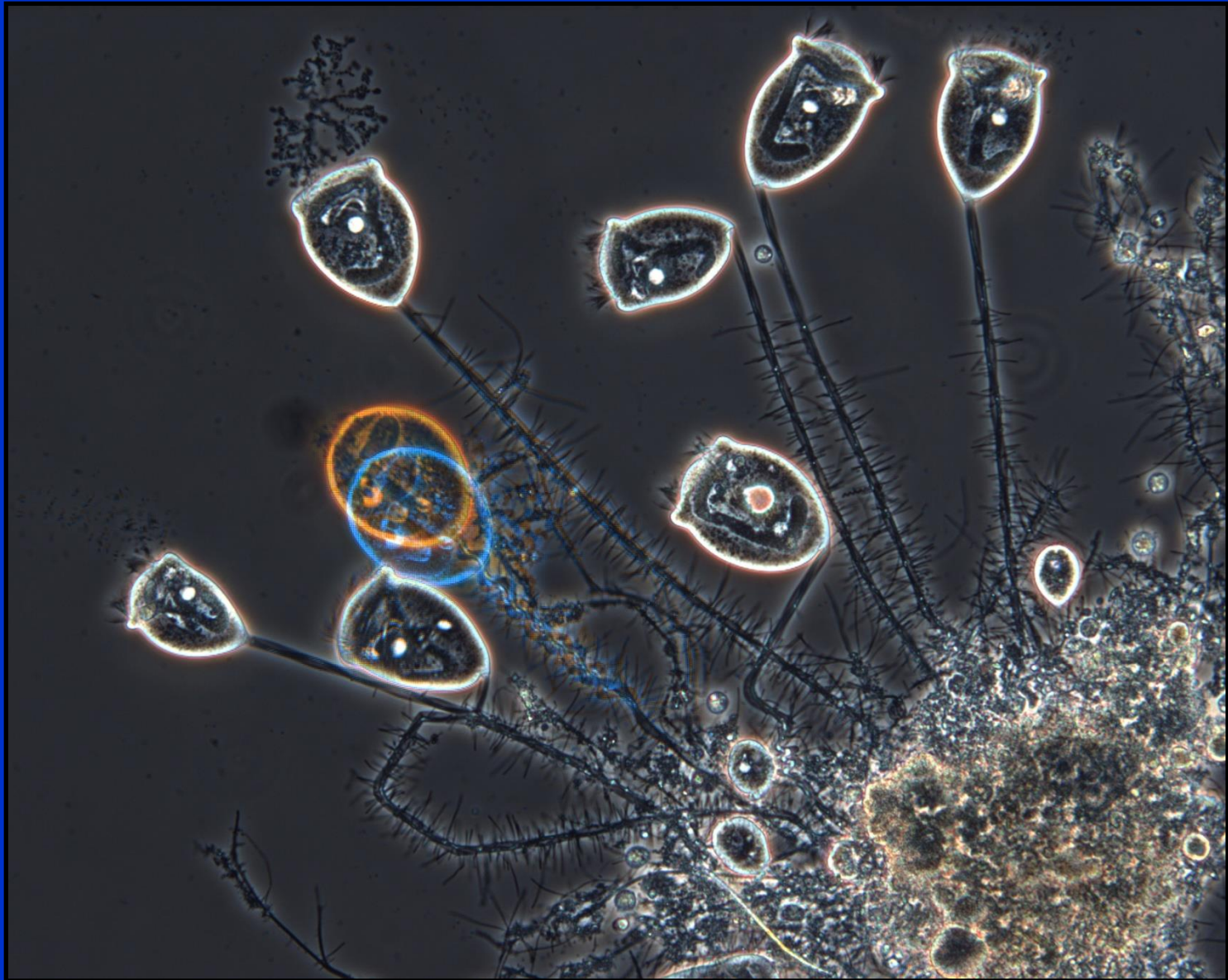
Crawling Ciliates



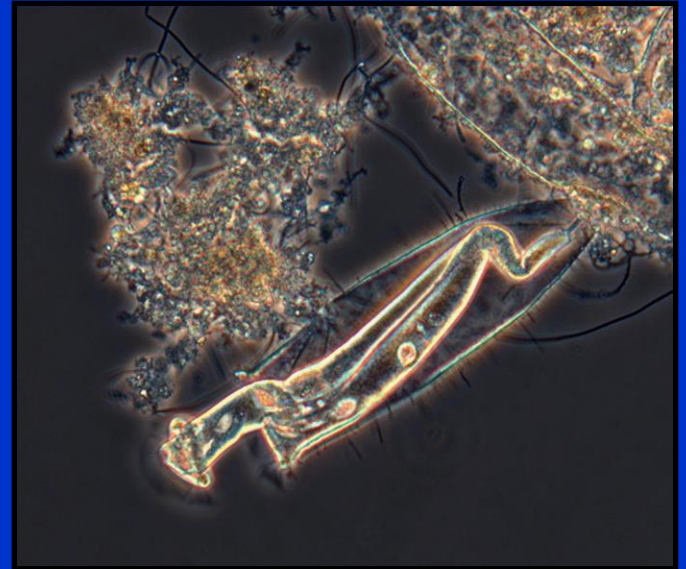
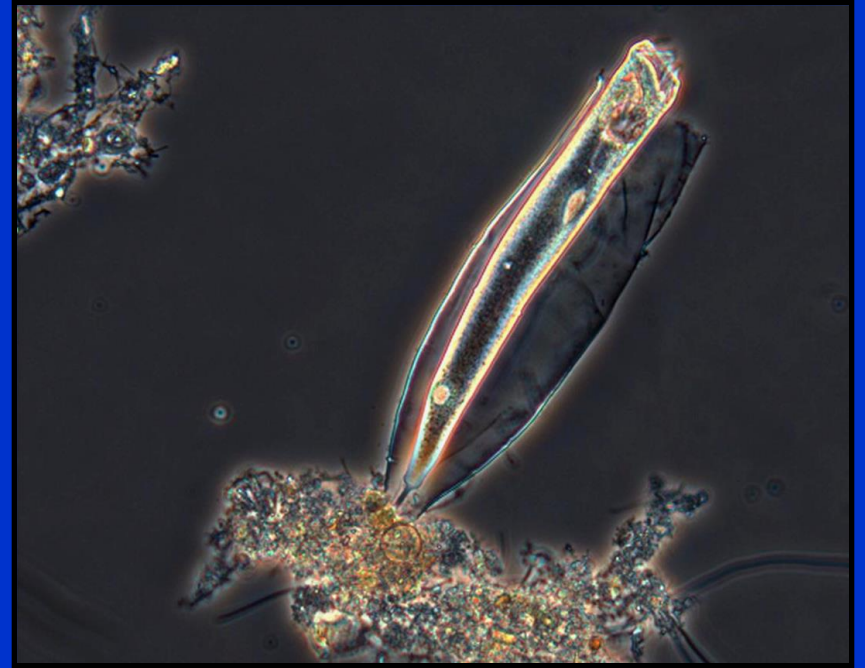
Stalked Ciliates



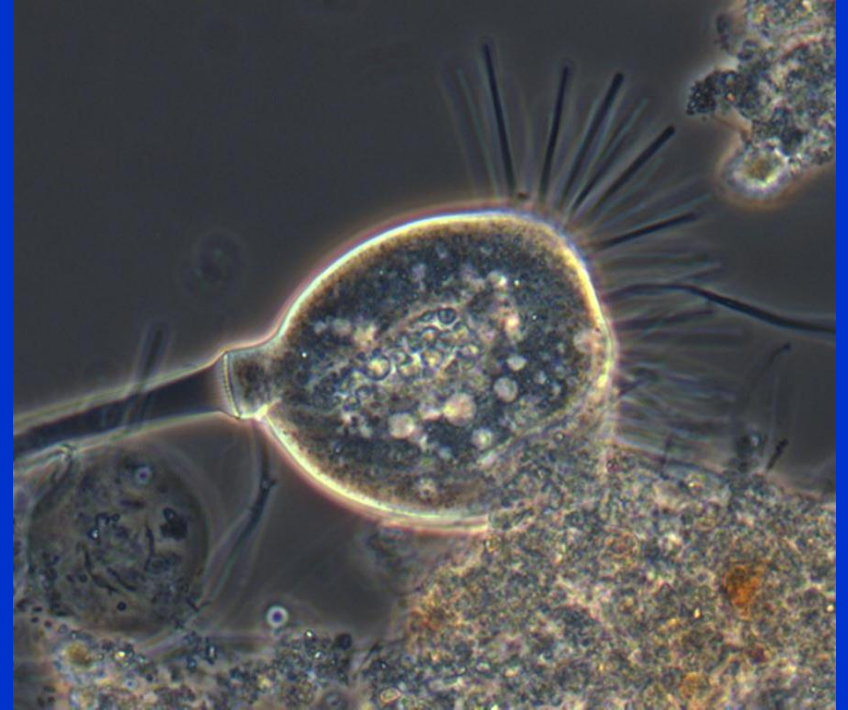
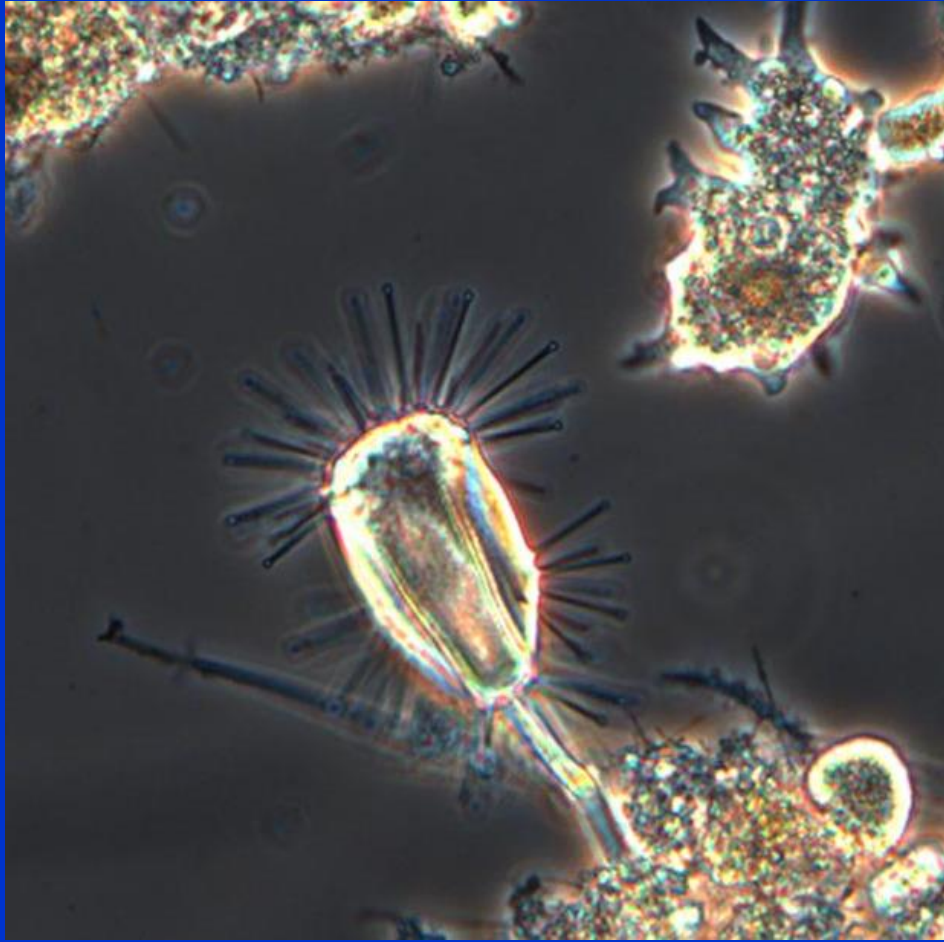
Stalked Ciliates



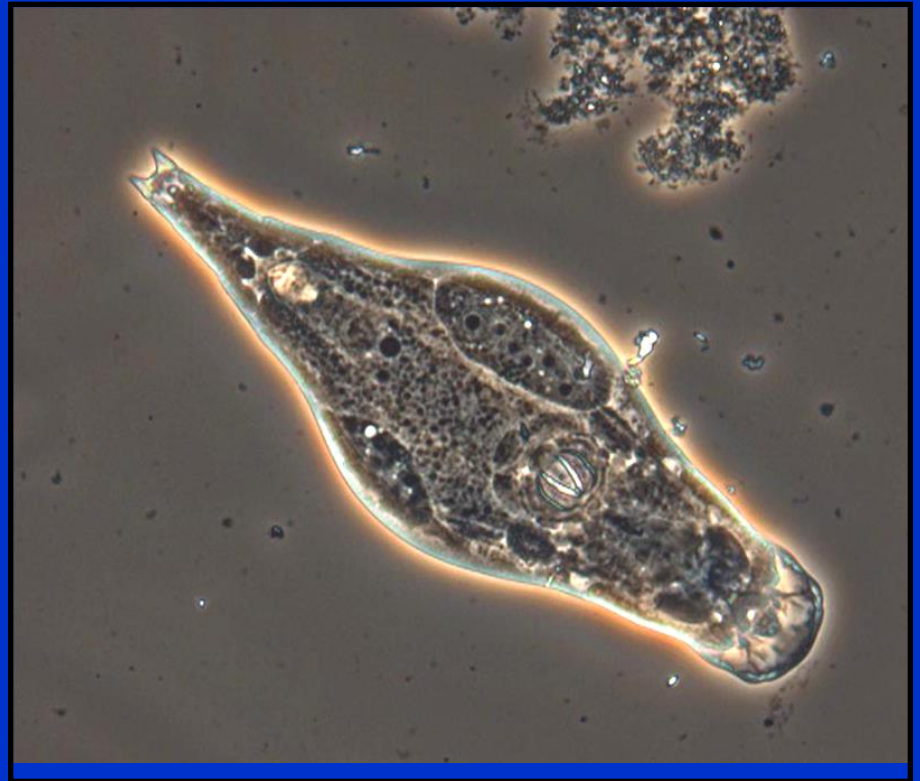
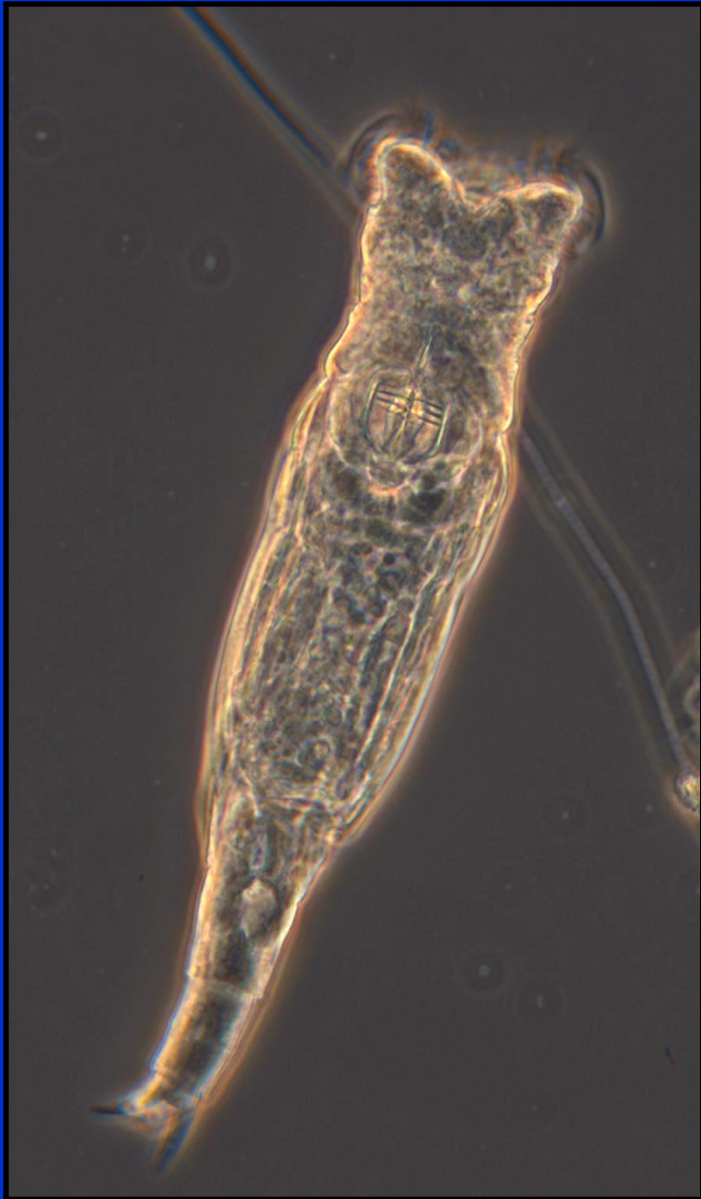
Vaginicola



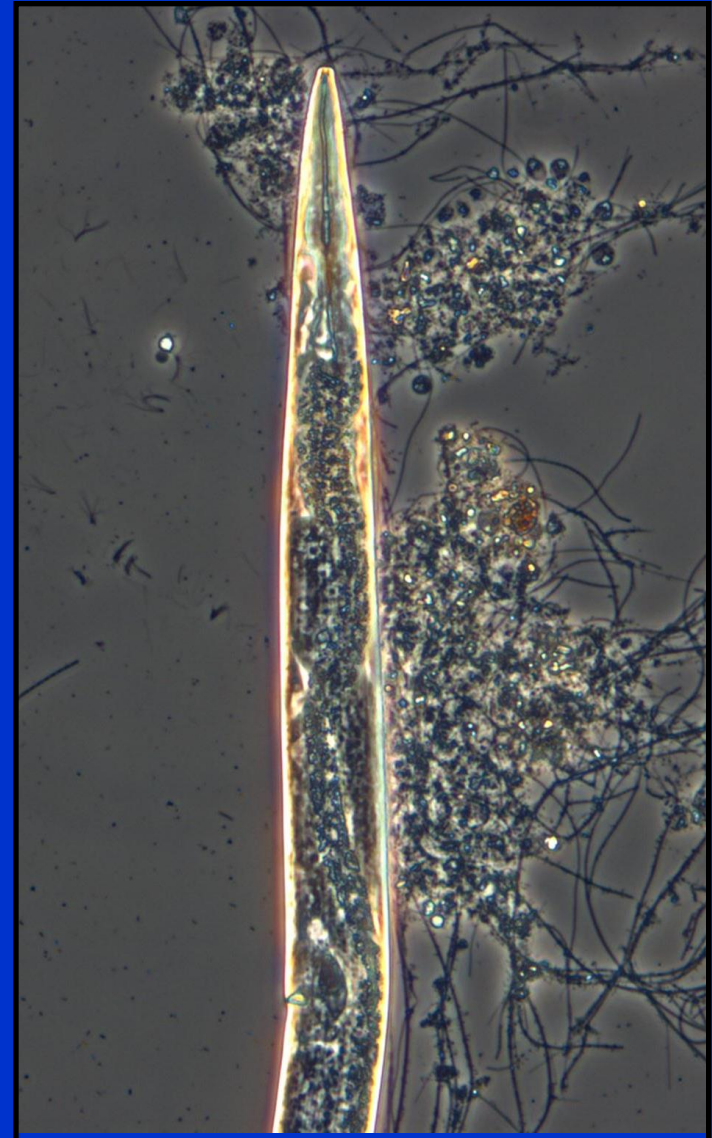
Suctororia



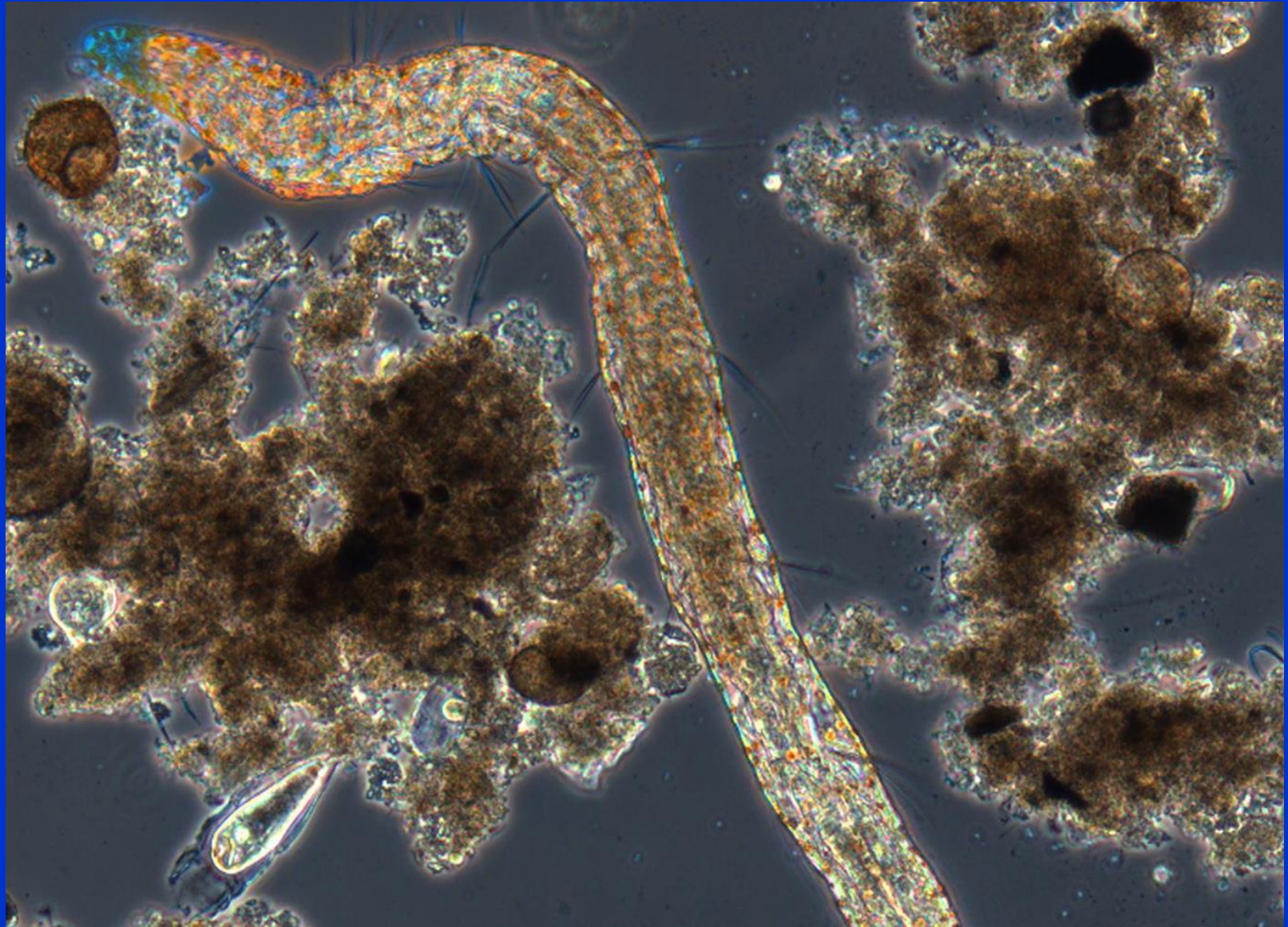
Rotifers



Nematodes



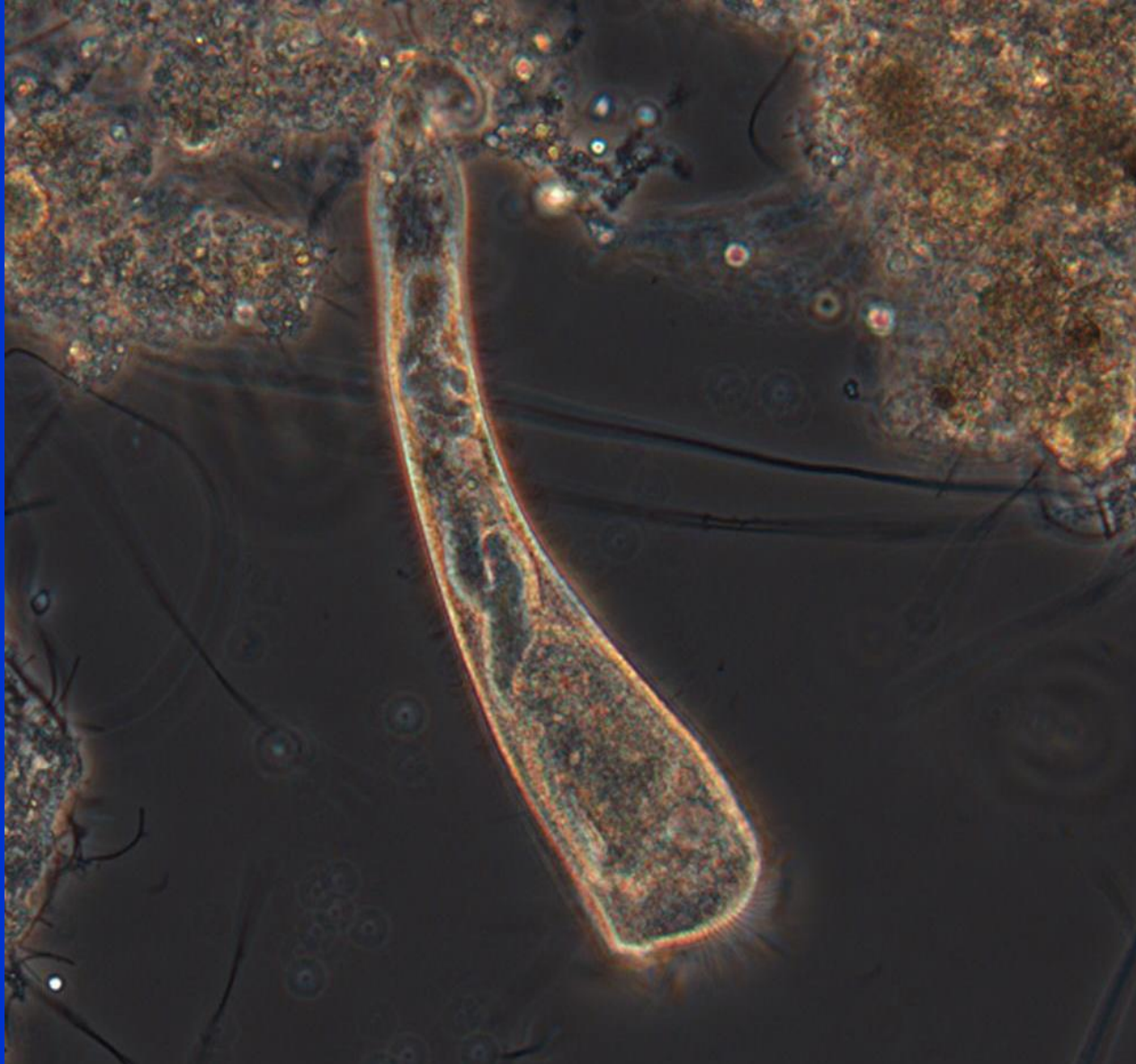
Bristleworm



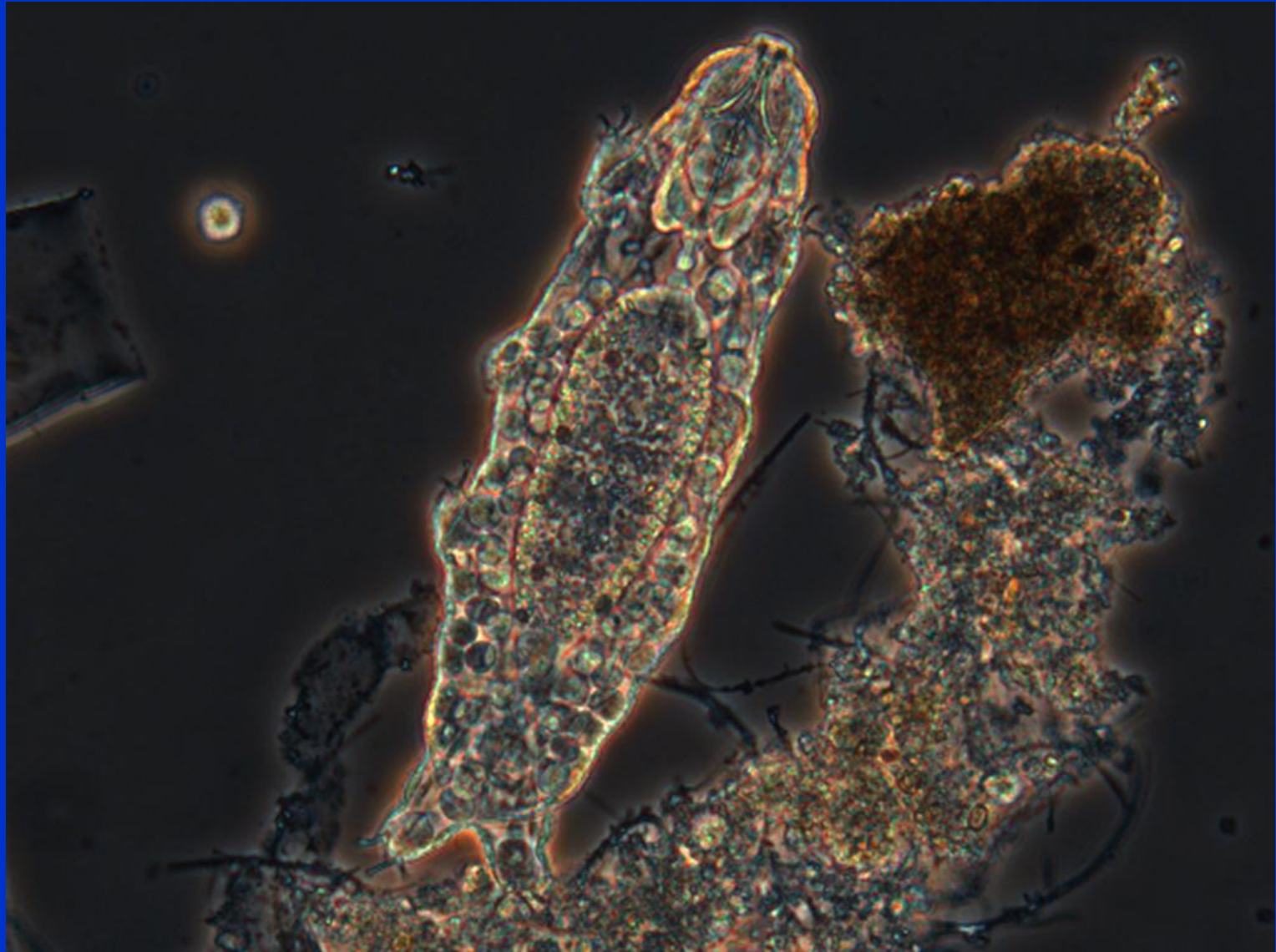
Paramecium



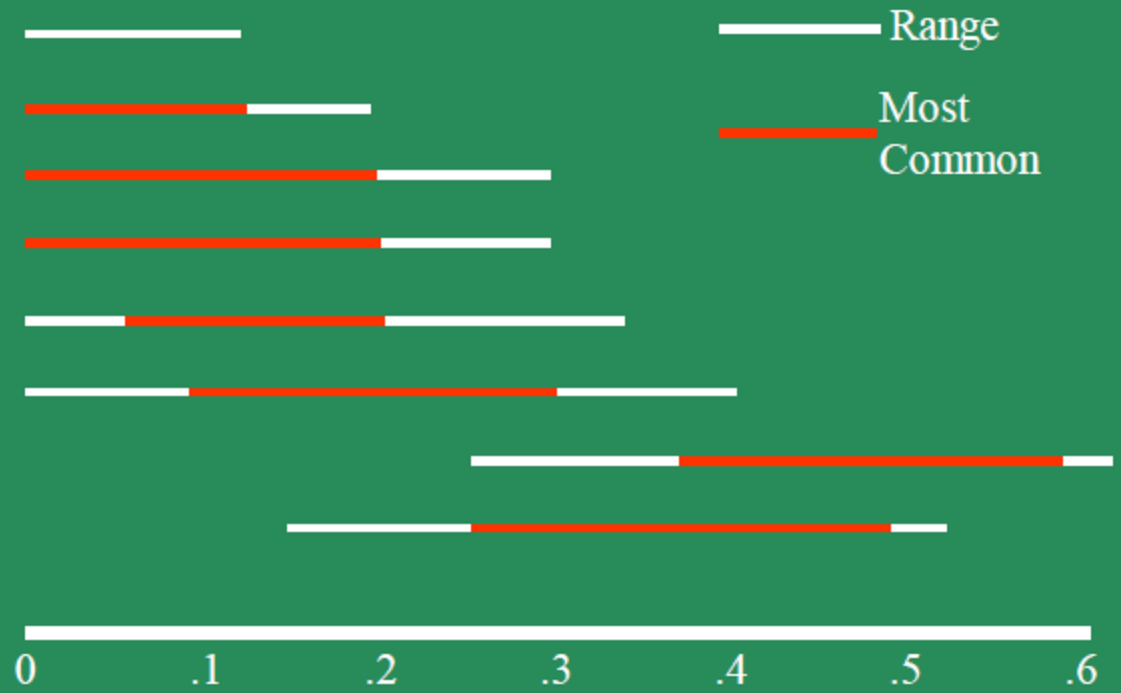
Stentor



Water Bear



Metazoa
Shelled Am.
Carnivores
Stalks
Crawlers
Free-Swim.
Flagellates
Amoeba

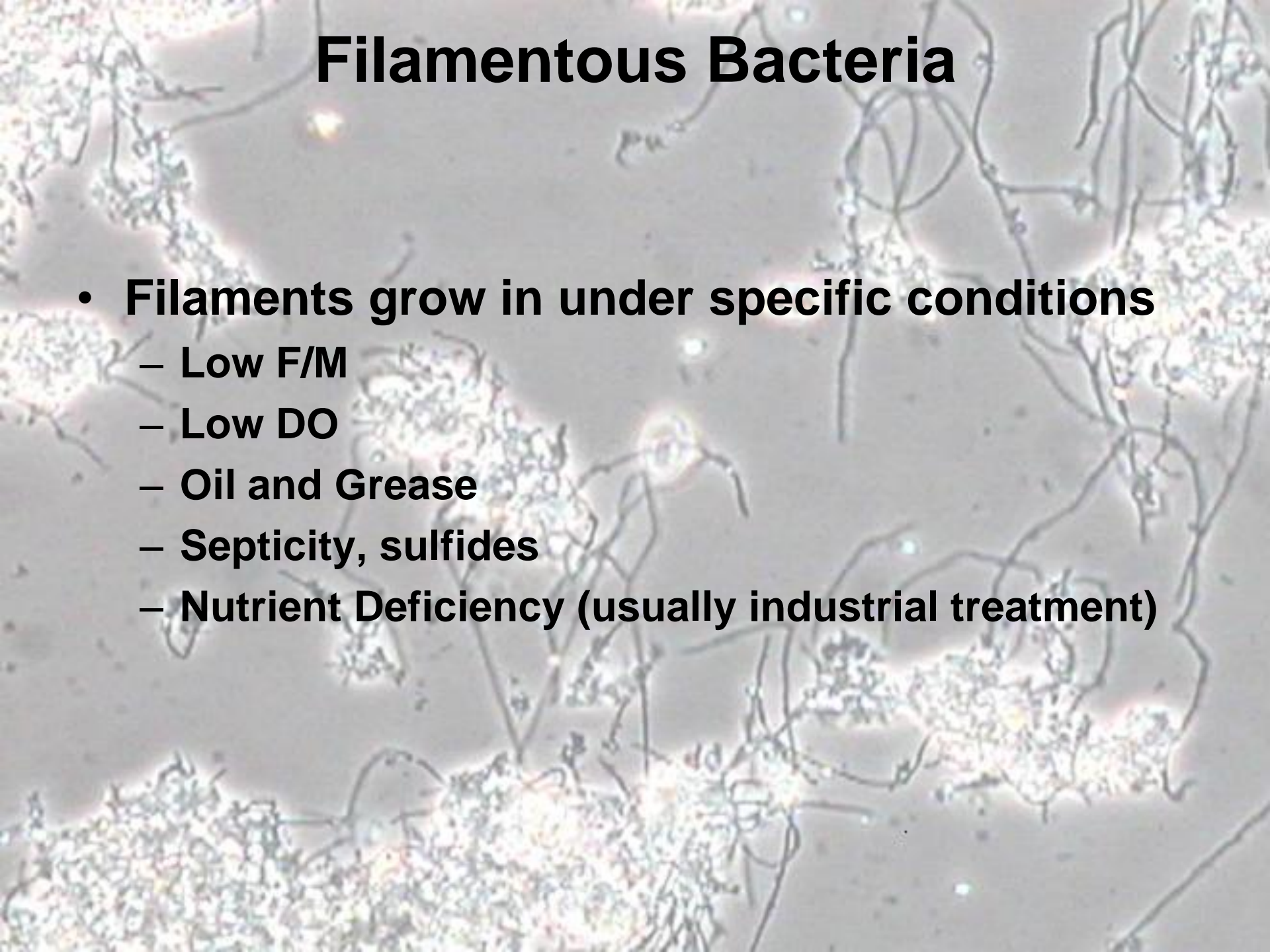


F/M lb BOD/lb MLSS (Eikelboom, 2000)

A light micrograph showing numerous long, thin, filamentous structures, likely bacteria, scattered across the field of view. The filaments are thin and thread-like, with some appearing to have small, dark, rounded structures attached to them. The background is a light, slightly grainy grey. The text "The Filamentous Bacteria" is overlaid in the center in a bold, black, sans-serif font.

The Filamentous Bacteria

Filamentous Bacteria

A microscopic image showing numerous long, thin, filamentous structures, likely bacteria, against a light background. The filaments are tangled and some have small, dark, rounded structures attached to them, possibly spores or other cellular components.

- **Filaments grow in under specific conditions**
 - **Low F/M**
 - **Low DO**
 - **Oil and Grease**
 - **Septicity, sulfides**
 - **Nutrient Deficiency (usually industrial treatment)**

Filamentous Bacteria Commonly Found in WWTPs

Low F/M:

Type 0041

Type 0675

Type 1851

Type 0803

Oil and Grease:

Microthrix parvicella

Nocardia spp.

Type 1863

Low DO:

Sphaerotilus natans

Type 1701

Haliscomenobacter hydrossis

Septicity:

Type 021N

Thiothrix I and II

Beggiatoa

Type 0961

Type 0581

Type 0411

Type 0092

Nostocoida limicola I, II, and III

Type 0914

Nutrient Deficiency:

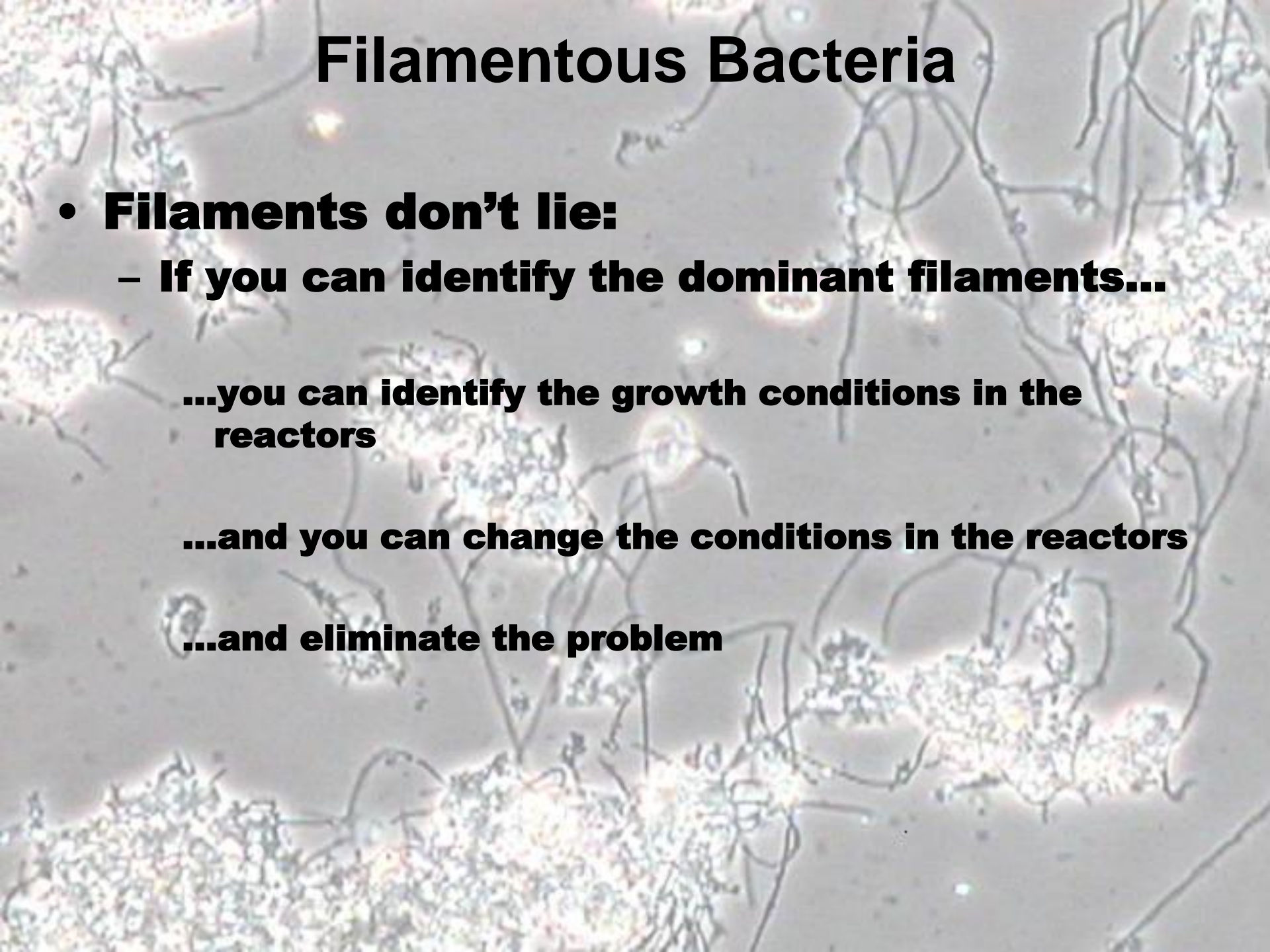
Type 021N

Thiothrix I and II

Nostocoida limicola III

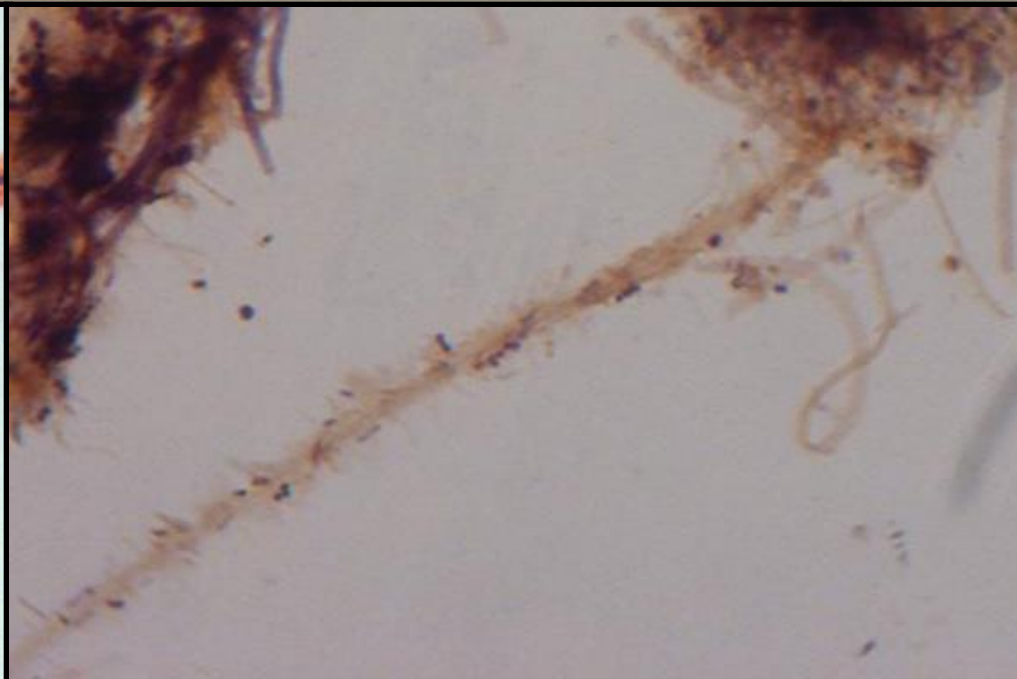
Haliscomenobacter hydrossis

Filamentous Bacteria

A microscopic image showing numerous long, thin, filamentous structures, likely bacteria, against a light background. The filaments are tangled and vary in length and thickness, some appearing as thin lines while others are thicker and more clumped.

- **Filaments don't lie:**
 - **If you can identify the dominant filaments...**
 - ...you can identify the growth conditions in the reactors**
 - ...and you can change the conditions in the reactors**
 - ...and eliminate the problem**

Type 0041

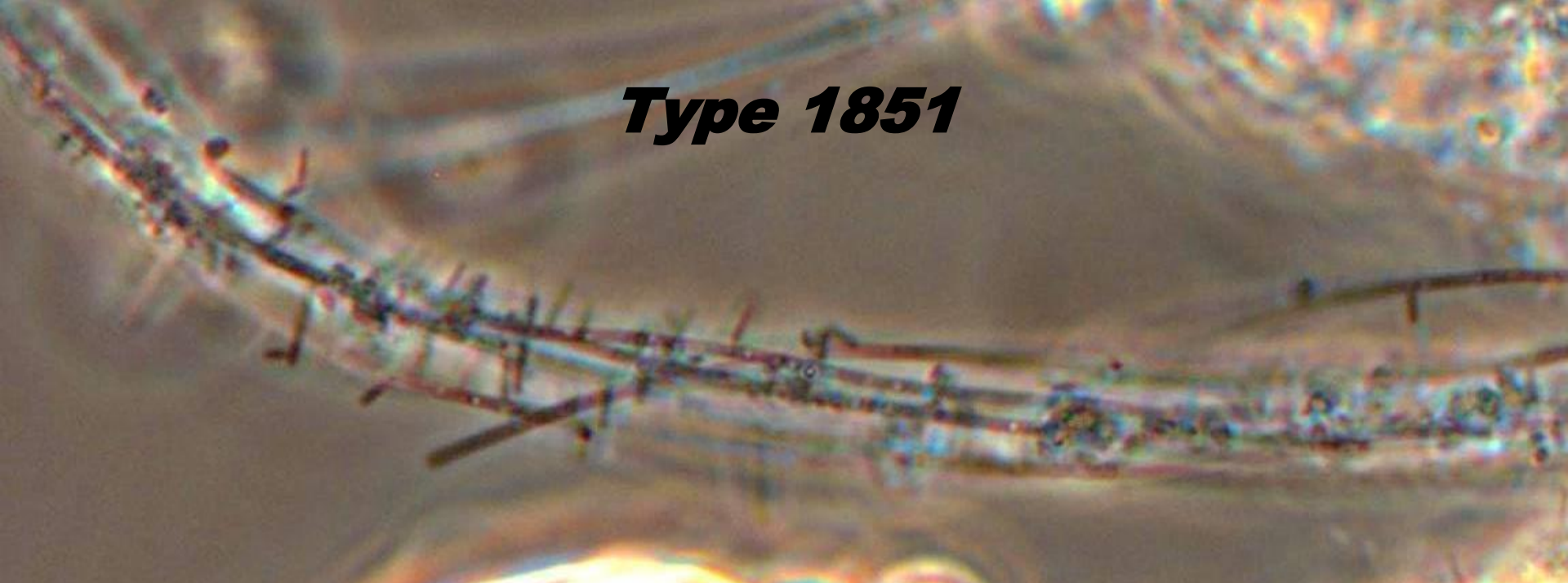


Type 0041

- **Growth Conditions:**
 - Low F/M
 - Slowly degradable (particulate) BOD
- **Response:**
 - Increase Wasting

(Note: Neisser negative difficult to see)

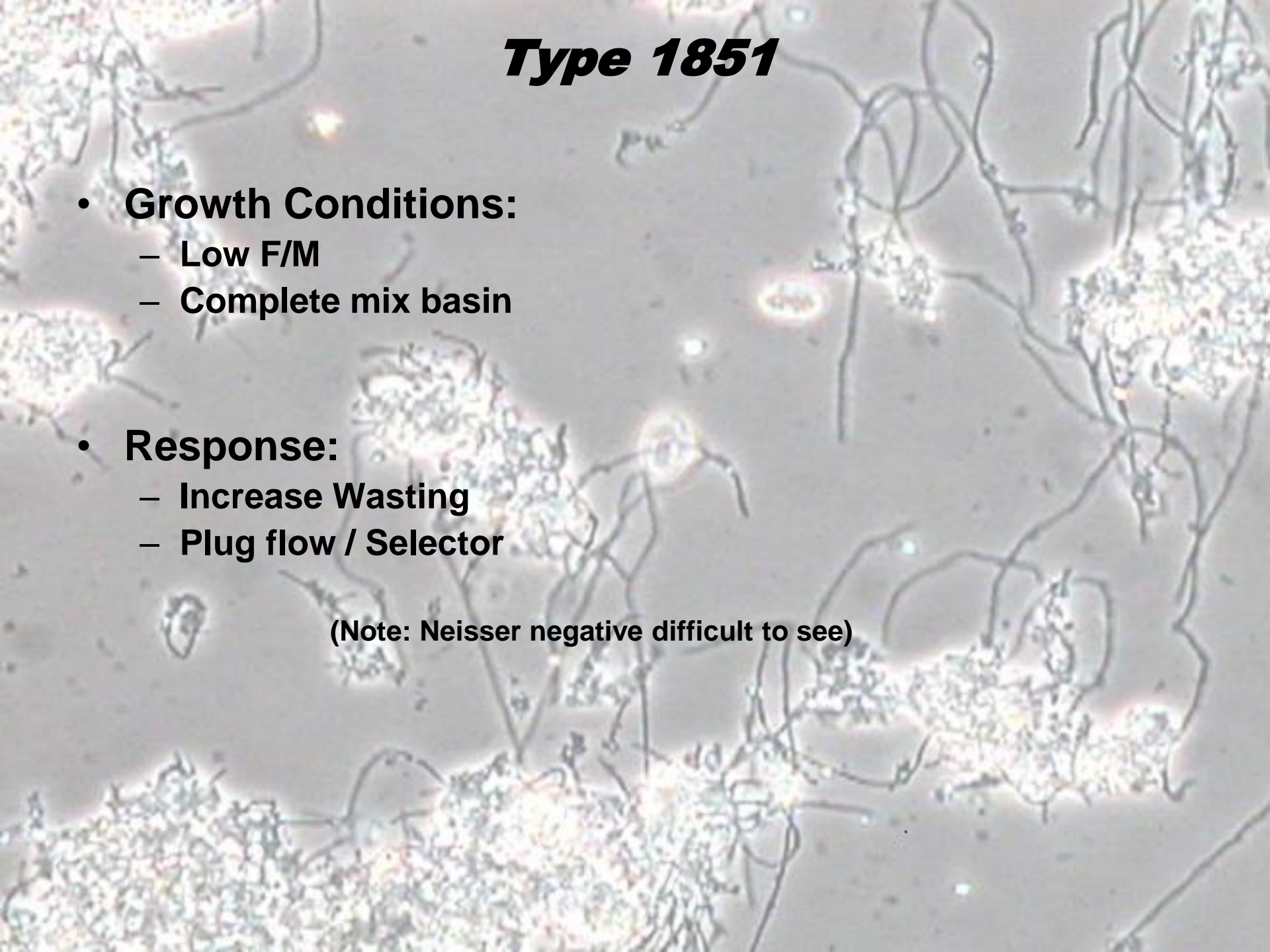
Type 1851



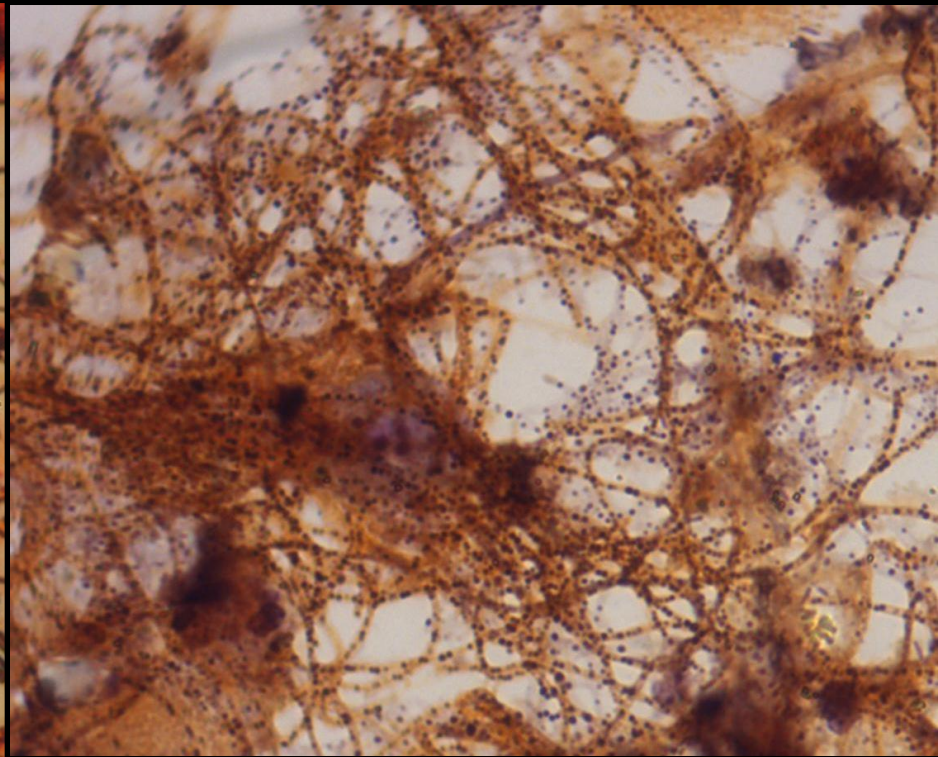
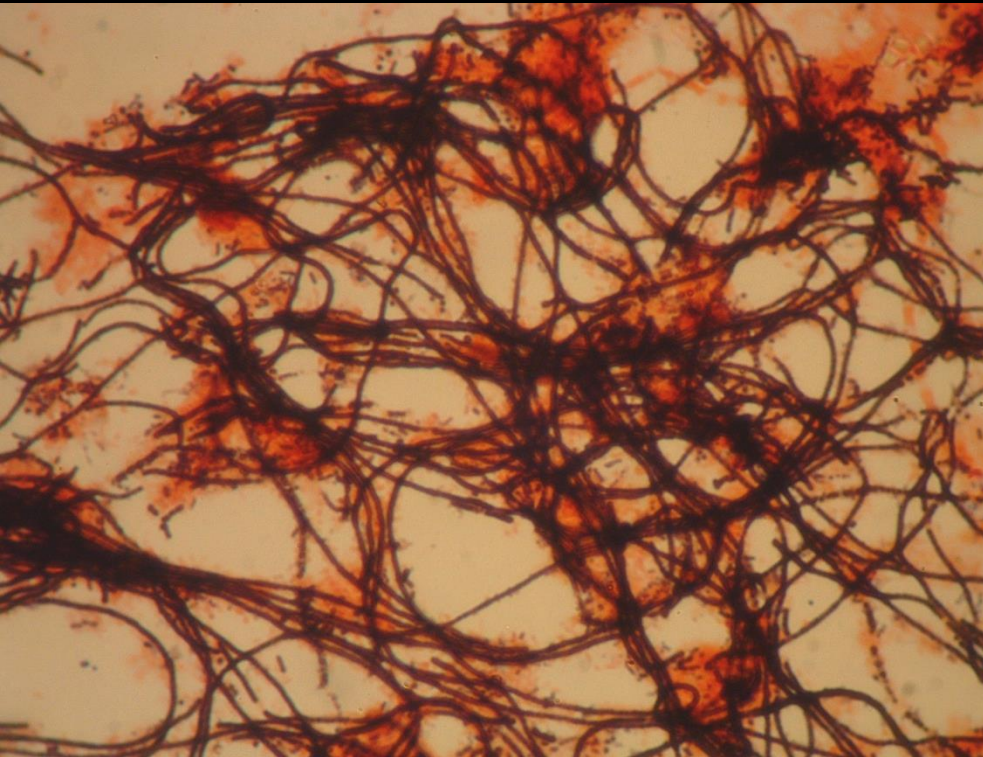
Type 1851

- **Growth Conditions:**
 - Low F/M
 - Complete mix basin
- **Response:**
 - Increase Wasting
 - Plug flow / Selector

(Note: Neisser negative difficult to see)



Microthrix Parvicella

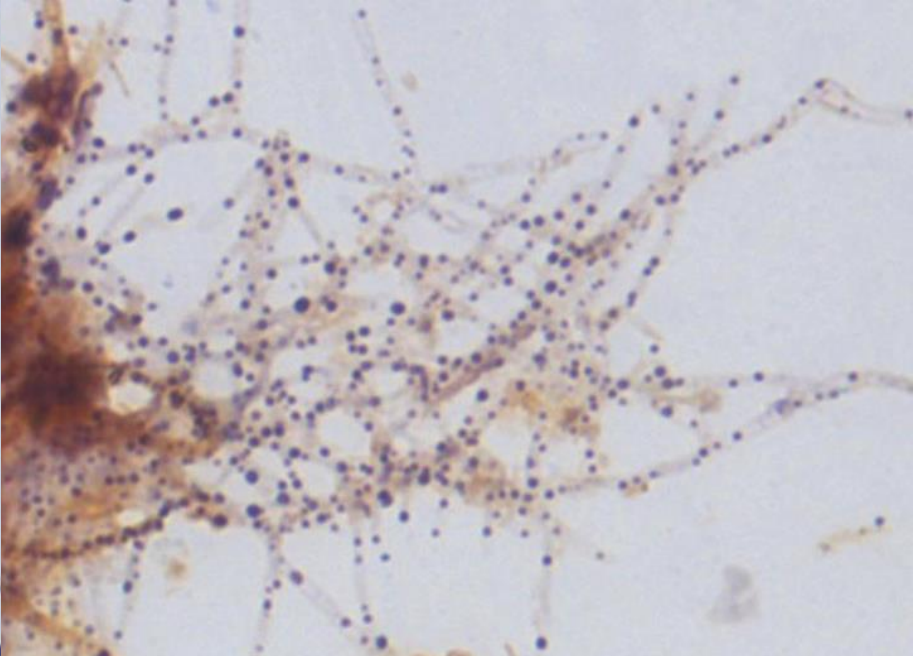
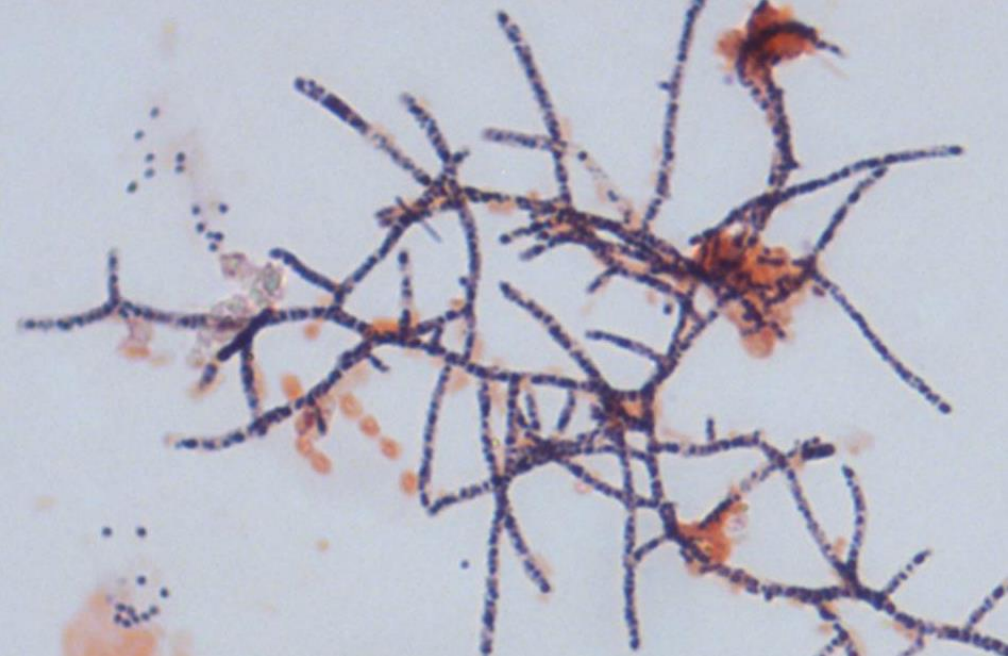
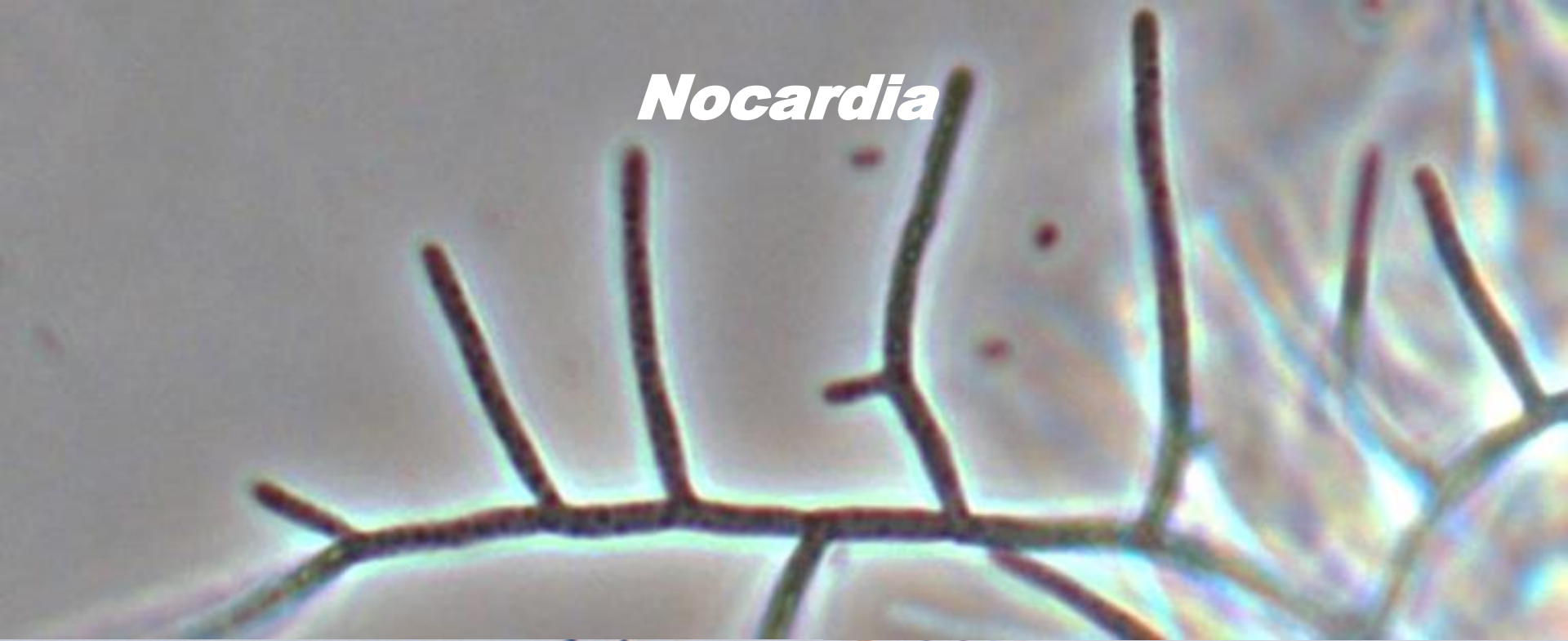


Microthrix Parvicella

- **Growth Conditions:**
 - Oil and Grease (lipids); High Carbon Chain Fatty Acids
 - Low F/M
 - Low DO
 - Cold water temperature
- **Response:**
 - Oil and Grease control (primary clarifier)
 - Foam trapping eliminated
 - Increase Wasting
 - Maintain adequate DO

(Note: Neisser positive granules occur)

Nocardia

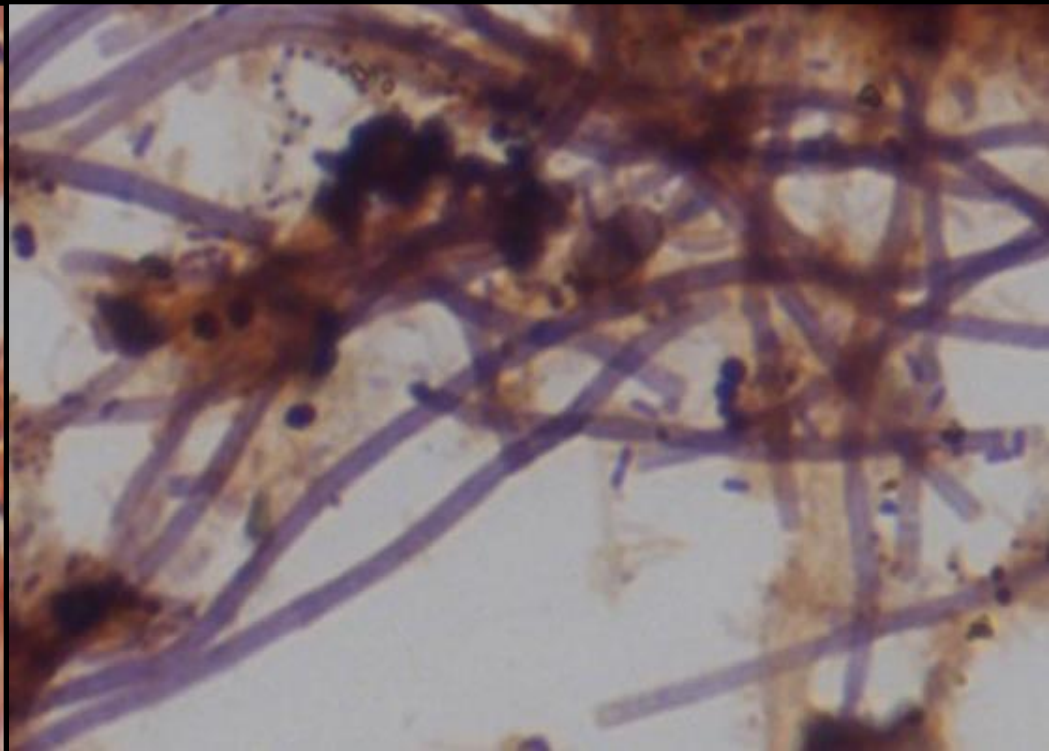


Nocardia

- **Growth Conditions:**
 - Fats, Oil and Grease (lipids)
 - Foam trapping
 - Lower organic loading (Low F/M environment)
 - Low aeration tank pH
- **Response:**
 - Oil and Grease control (primary clarifier)
 - Foam trapping eliminated
 - Waste...a lot

(Note: Neisser positive granules occur)

Type 0092

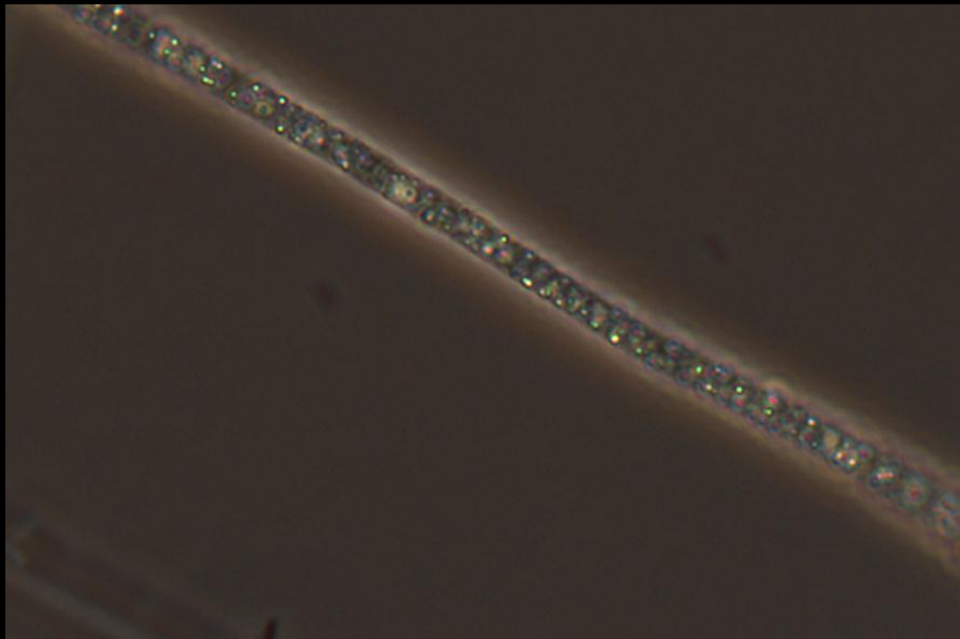


Type 0092

- **Growth Conditions:**
 - Septicity
 - Breakdown of biomass (clarifier full of solids?)
 - Low F/M
- **Response:**
 - Optimize clarifier operation
 - Optimize digester operation
 - Increase wasting

(Note: Gram negative difficult to see)

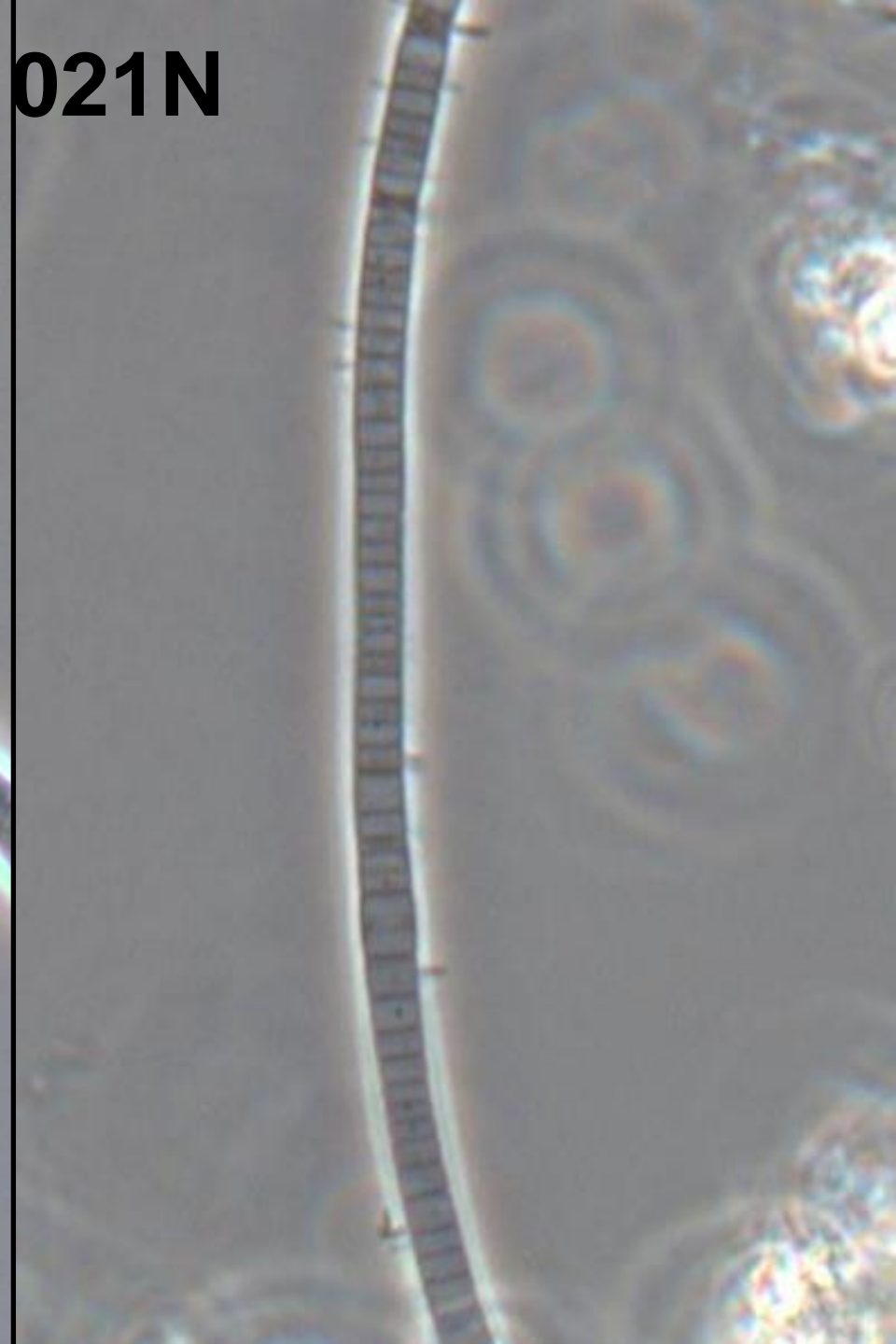
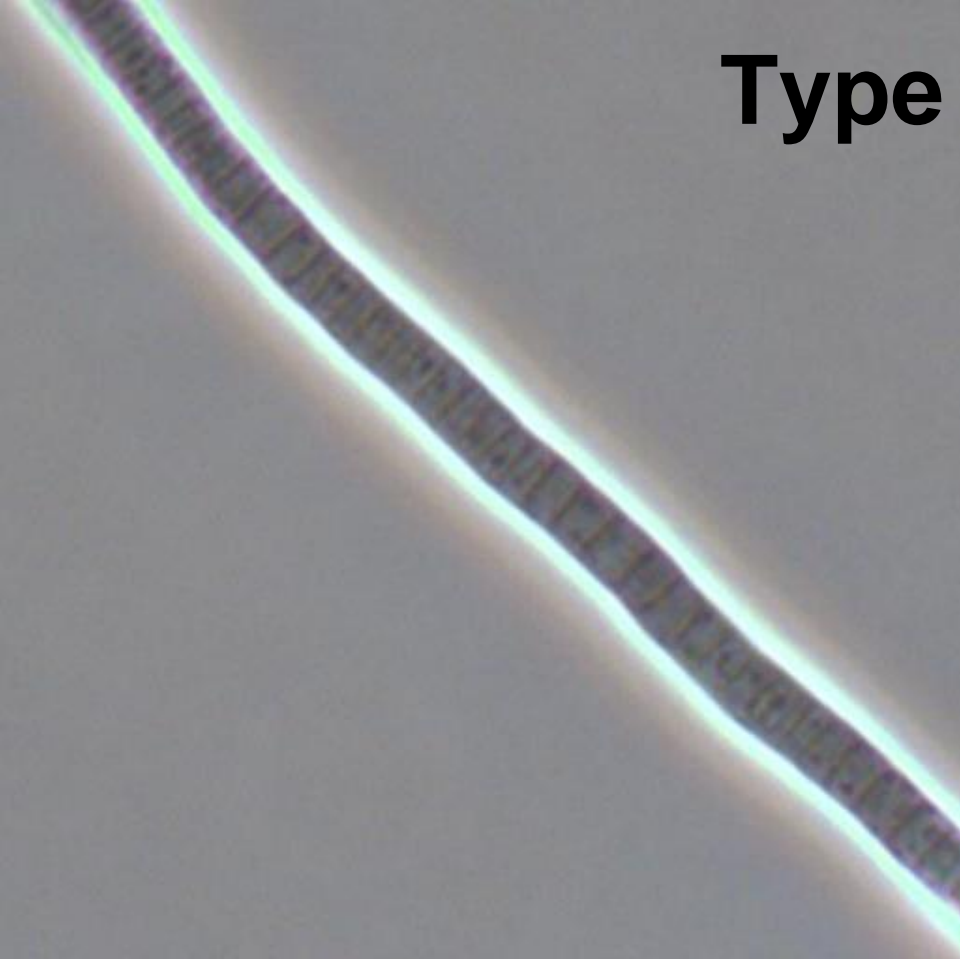
Thiothrix



Thiothrix

- **Growth Conditions:**
 - Septicity, low molecular weight organic acids
 - Sulfides
 - Typically higher F/M environment
- **Response:**
 - Remove sources of septicity (long forcemains, excessive clarifier sludge blankets, digester decant)
 - Preaeration

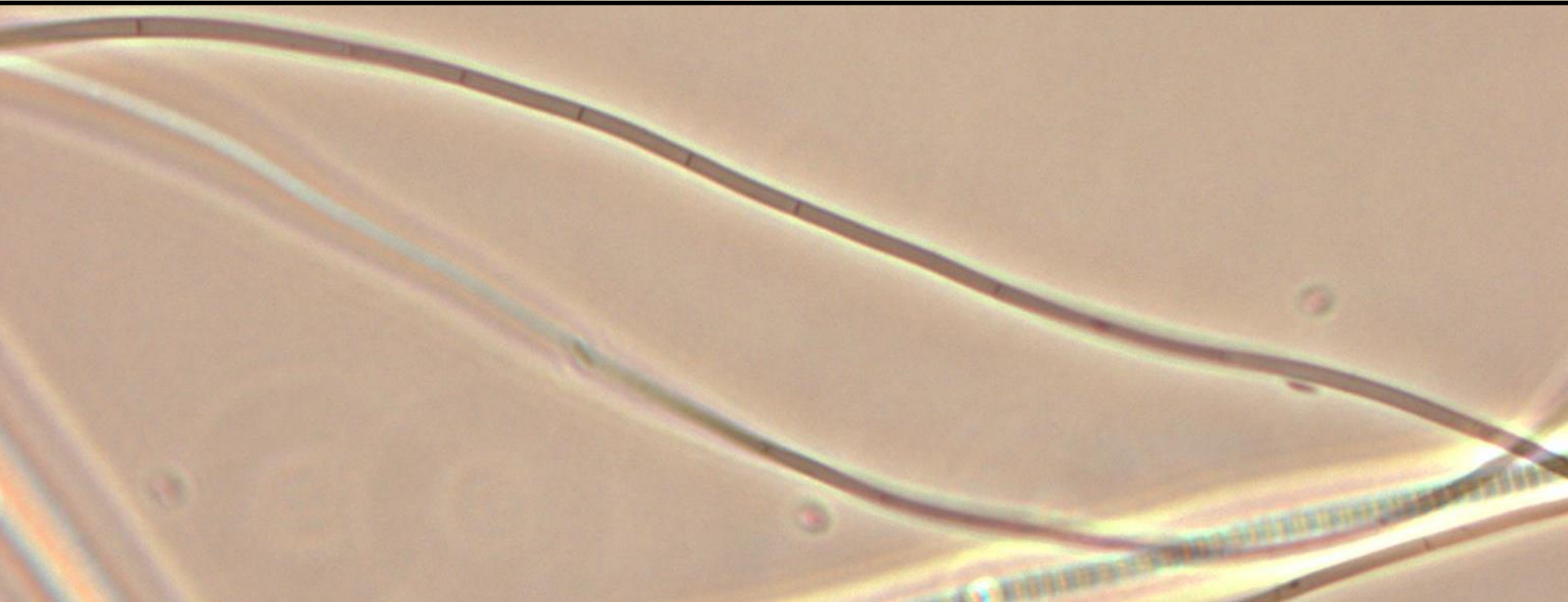
Type 021N



Type 021N

- **Growth Conditions:**
 - Septicity (low molecular weight organic acids)
 - Wide range of F/M
- **Response:**
 - Remove sources of septicity (long forcemains, excessive clarifier sludge blankets, digester decant)
 - Anoxic Selector

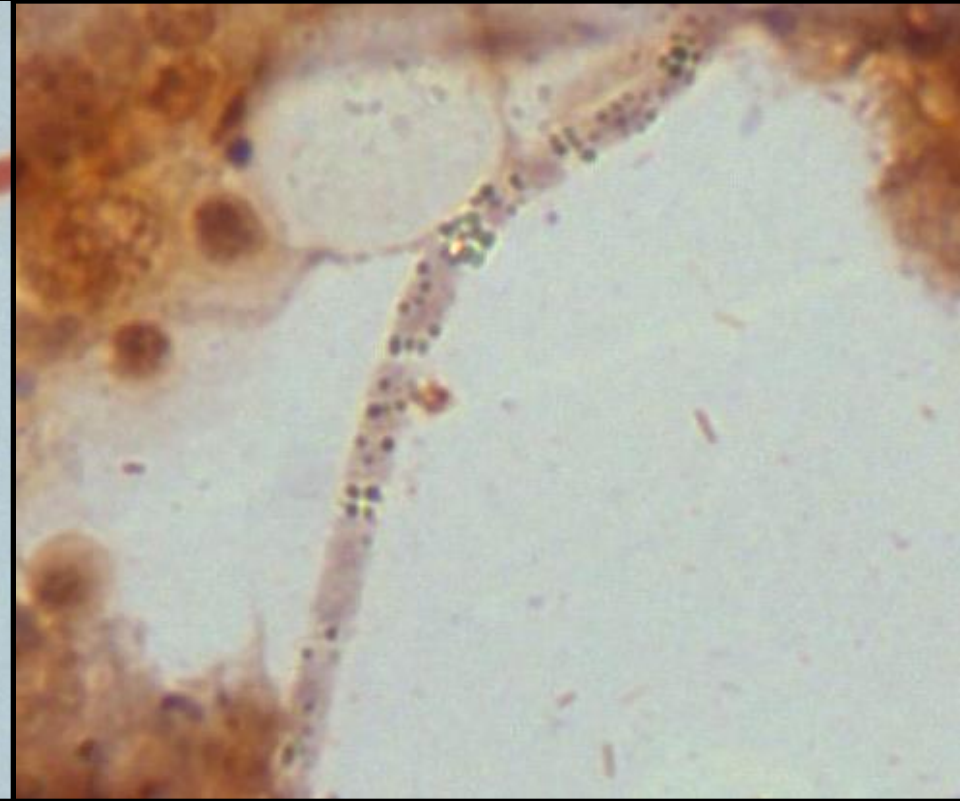
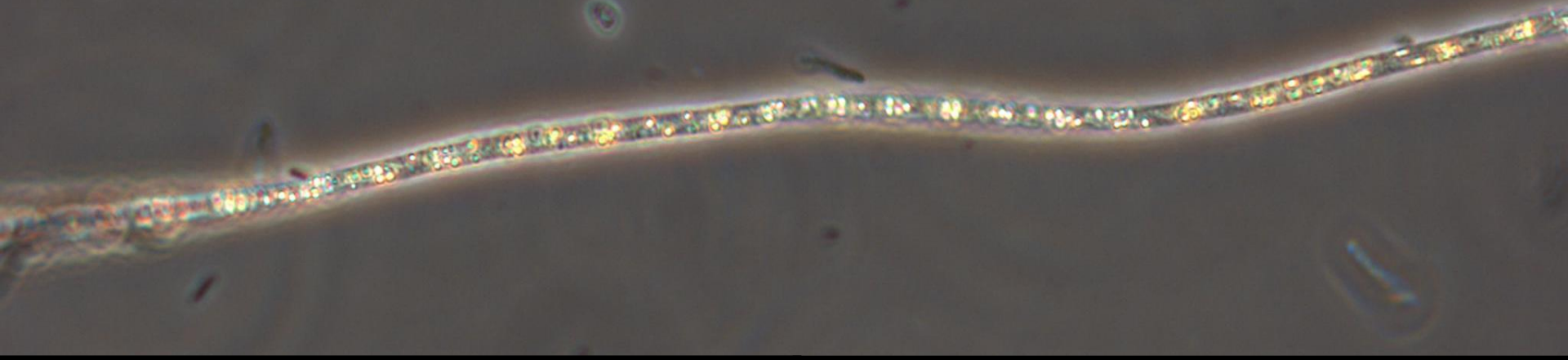
Type 0961



Type 0961

- **Growth Conditions:**
 - **Septicity (low molecular weight organic acids)**
 - **Lower F/M**
 - **Not very common in domestic wastewater**
- **Response:**
 - **Remove sources of septicity**
 - **Decrease MCRT (waste)**

Beggiatoa

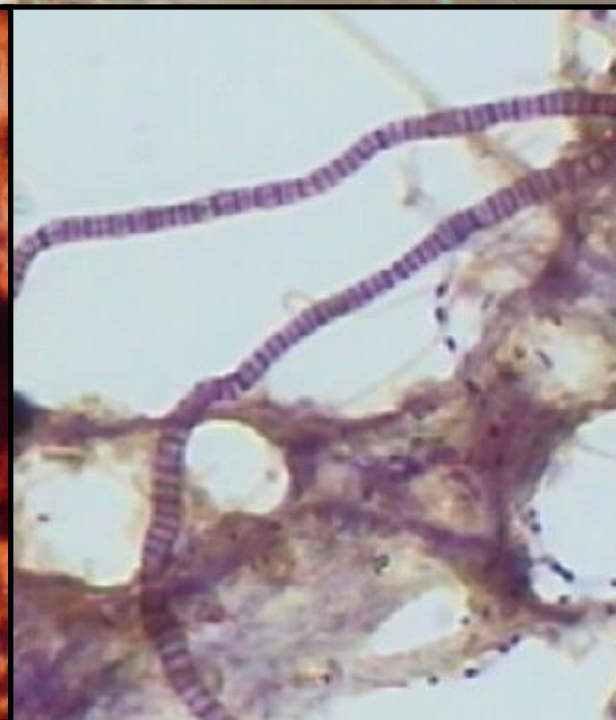
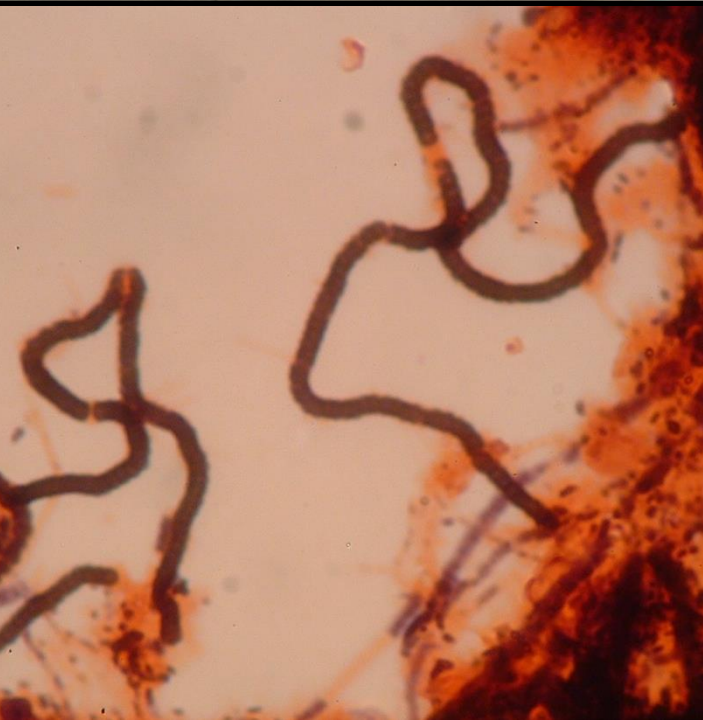


Beggiatoa



- **Growth Conditions:**
 - Sulfides in wastestream
 - Lower Dissolved Oxygen
- **Response:**
 - Preaerate to remove hydrogen sulfides in wastestream

Nostocoida Limicola

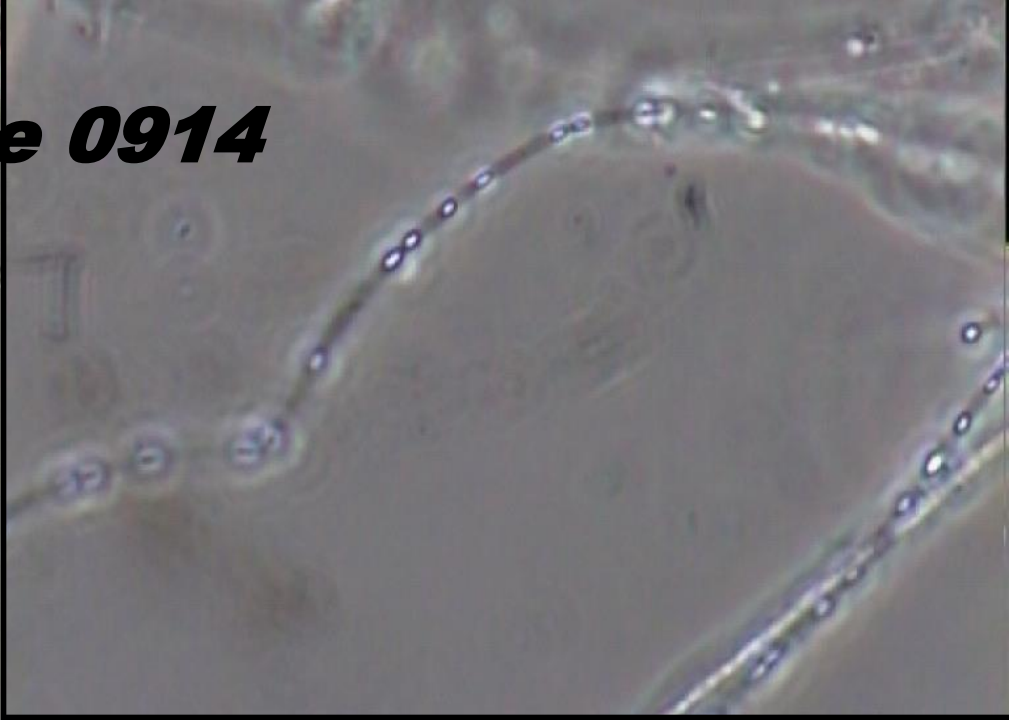


Nostocoida Limicola

- **Growth Conditions:**
 - **Septicity (low molecular weight organic acids)**
 - **Wide range of F/M**
 - **Nutrient Deficiency**
- **Response:**
 - **Investigate source of septicity and organic acids**
 - **Digesters, force mains, food processing sources**

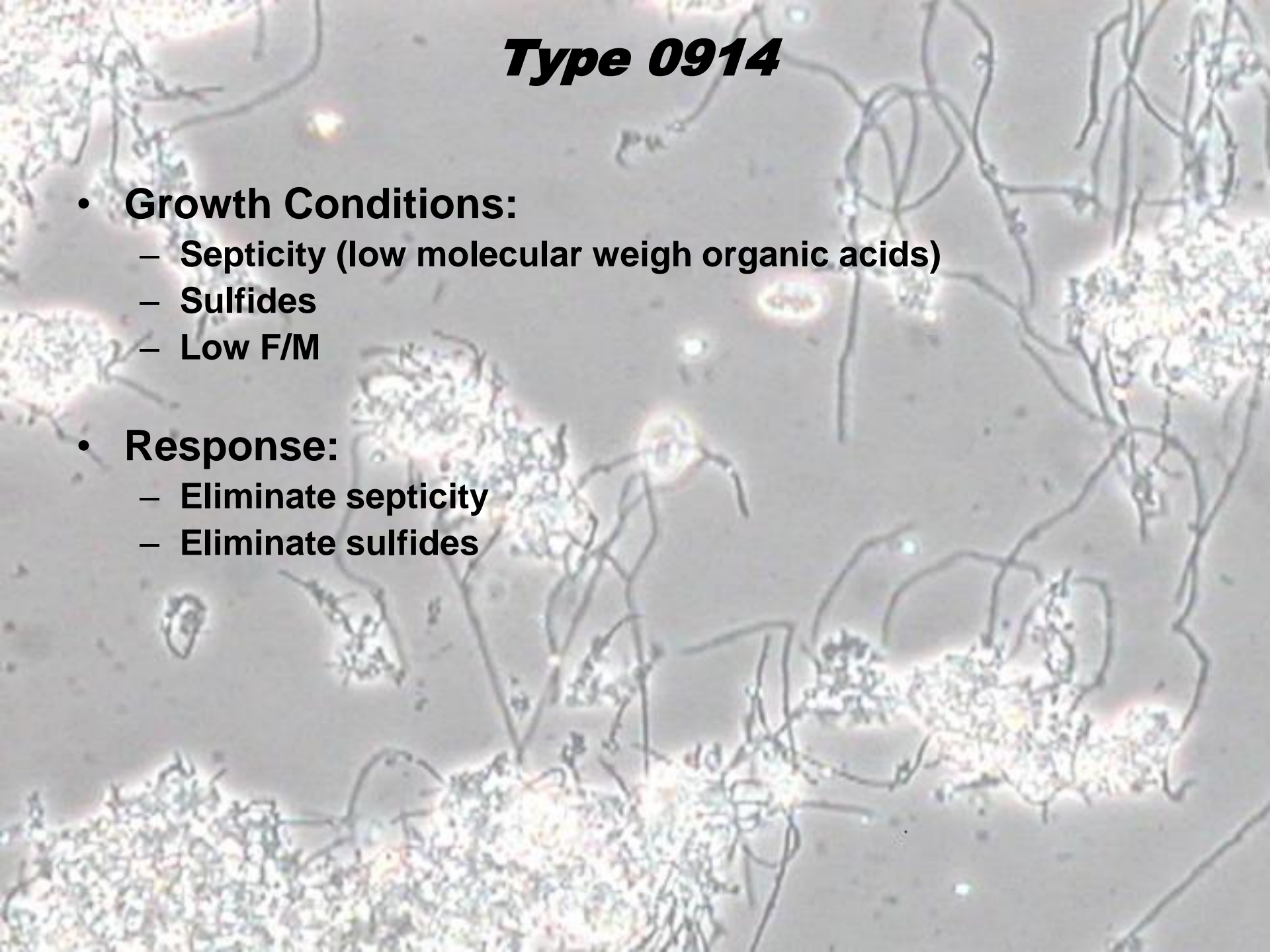
(Note: Gram and Neisser negative occur)

Type 0914

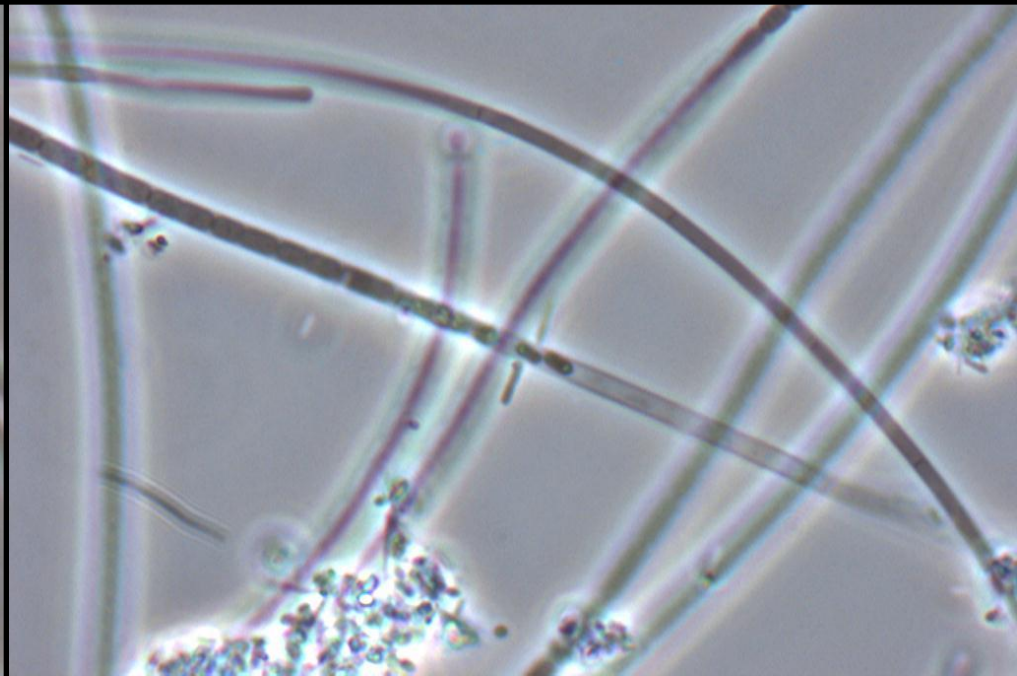


Type 0914

- **Growth Conditions:**
 - **Septicity (low molecular weight organic acids)**
 - **Sulfides**
 - **Low F/M**
- **Response:**
 - **Eliminate septicity**
 - **Eliminate sulfides**



Sphaerotilus natans

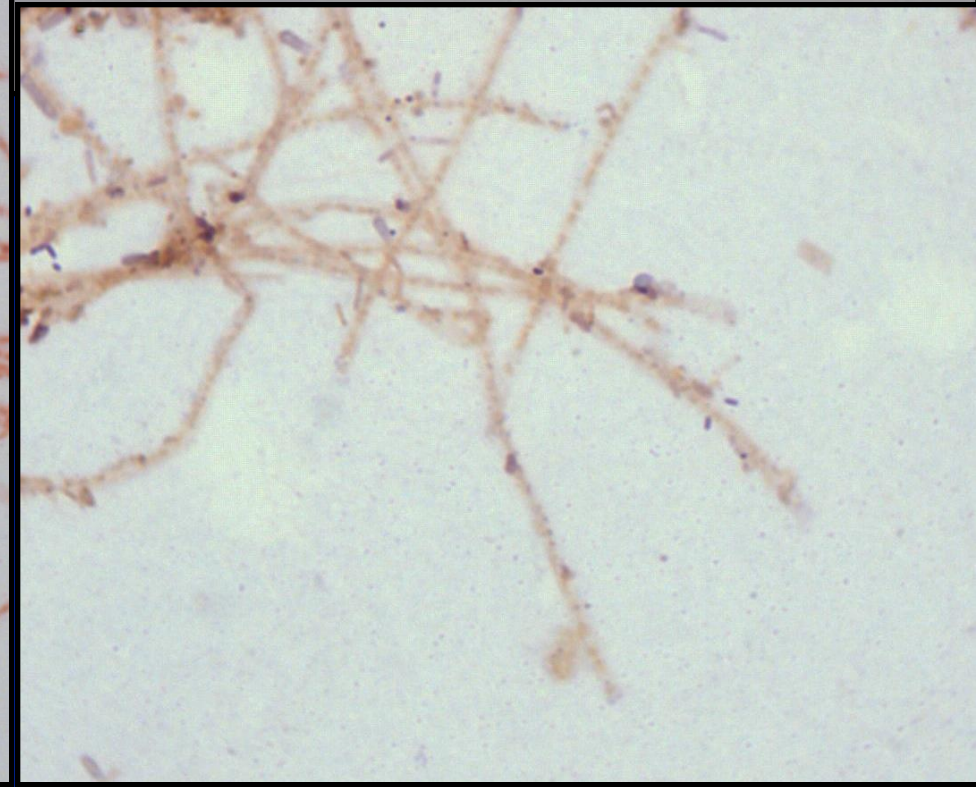
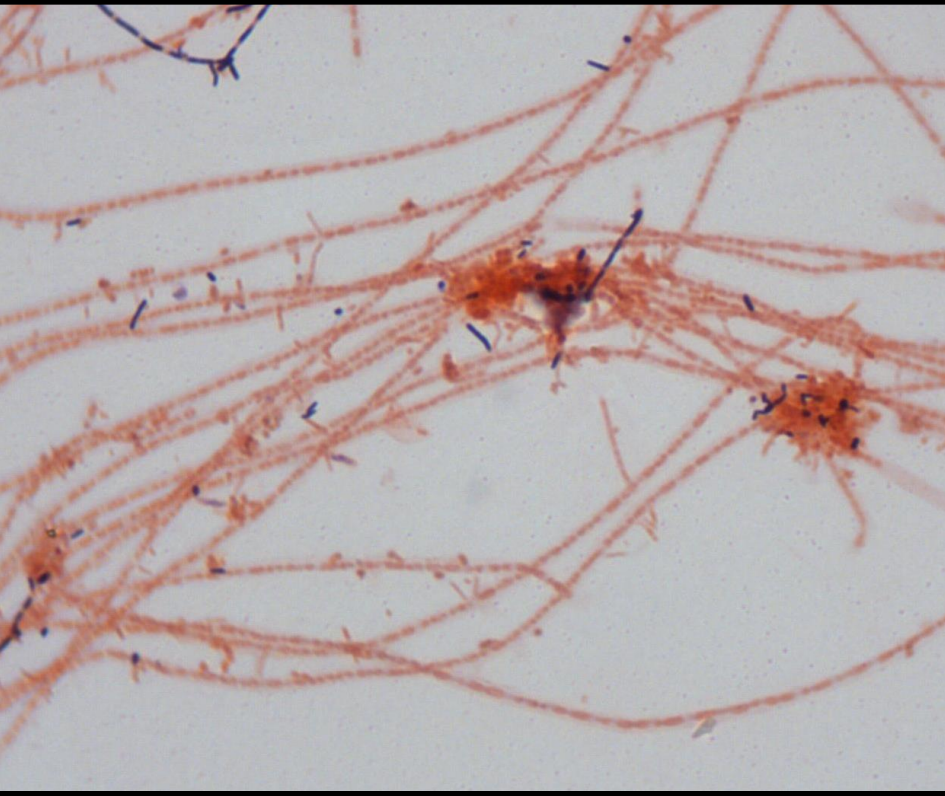
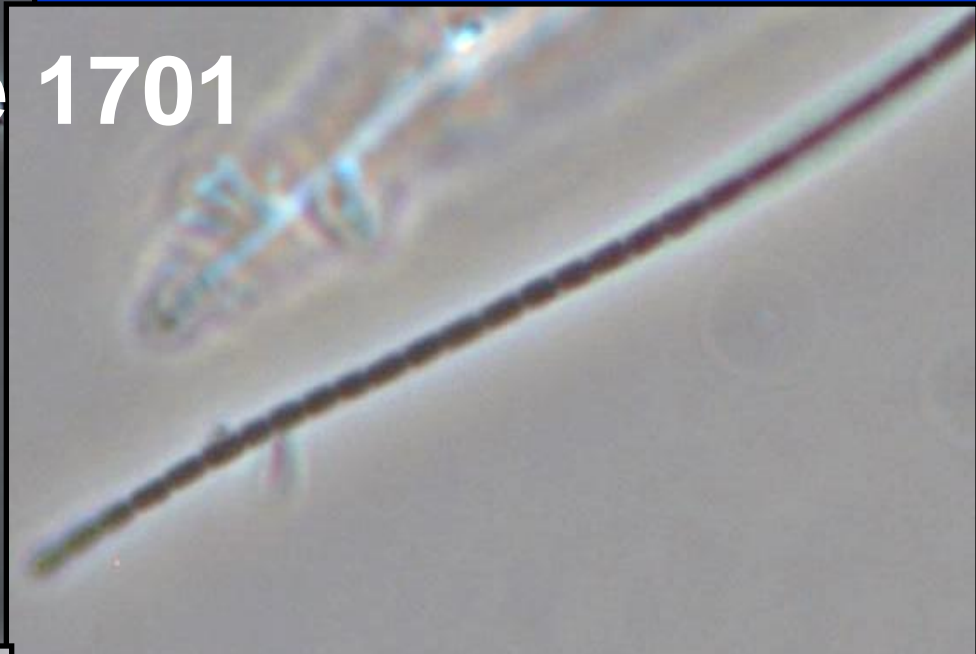
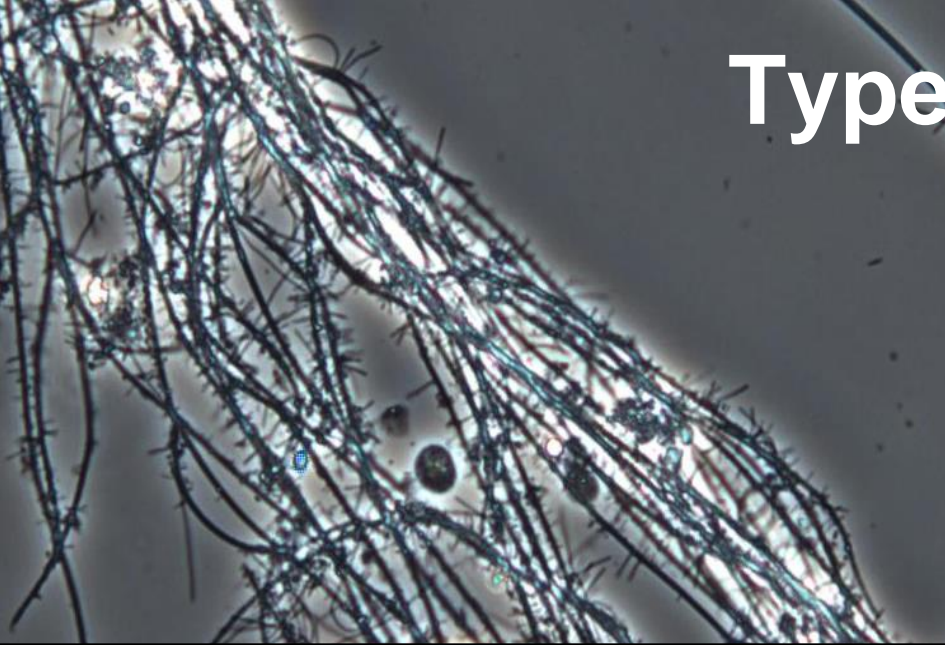


Sphaerotilus natans

A microscopic image showing numerous long, thin, filamentous structures of Sphaerotilus natans. The filaments are tangled and appear to be composed of individual cells or segments. Some filaments are straight, while others are curved or coiled. The background is a light, slightly grainy grey.

- **Growth Conditions:**
 - **Low Dissolved Oxygen**
 - Low DO for the applied load
 - Low DO in the interior of the floc
- **Response:**
 - **Increase bulk Dissolved Oxygen in Aeration**

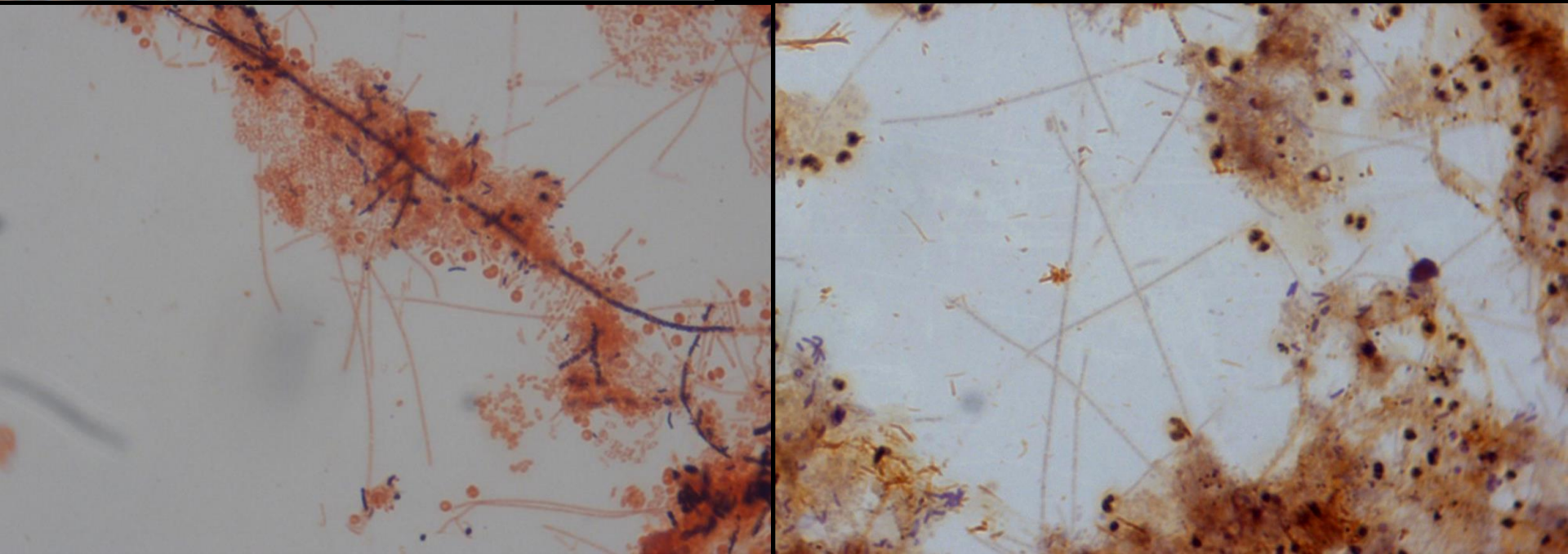
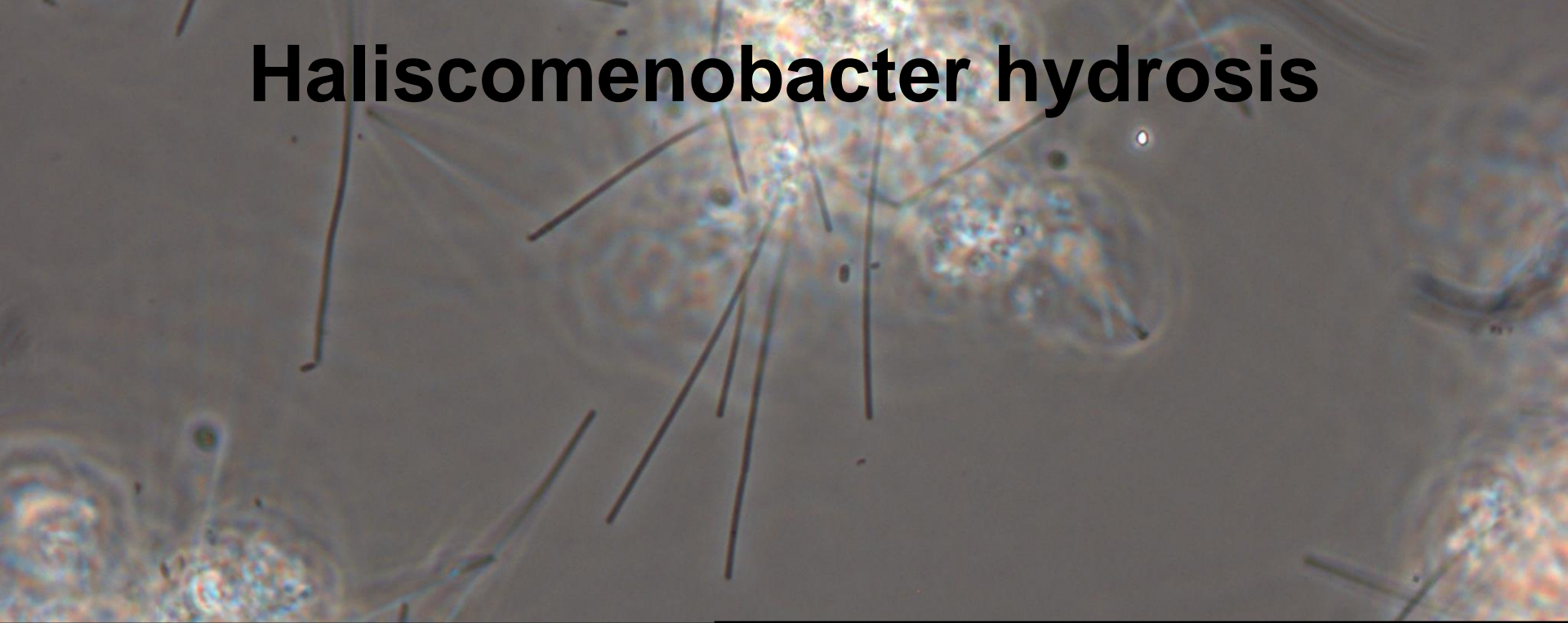
Type 1701



Type 1701

- **Growth Conditions:**
 - Low Dissolved Oxygen in Aeration Tank
 - Wide range of F/M
- **Response:**
 - Increase Dissolved Oxygen in Aeration Tank

Haliscomenobacter hydrosis



Haliscomenobacter hydrosis

- **Growth Conditions:**
 - Septicity
 - Low dissolved oxygen
 - High influent nitrogen (ammonia)
 - Wide range of F/M
- **Response:**
 - Remove sources of septicity (long forcemains, excessive clarifier sludge blankets, digester decant)
 - Increase dissolved oxygen in aeration tanks

22 Filamentous Bacteria Found in WWTPs

Low F/M:

Type 0041

Type 0675

Type 1851

Type 0803

Oil and Grease:

Microthrix parvicella

***Nocardia* spp.**

Type 1863

Low DO:

Sphaerotilus natans

Type 1701

Haliscomenobacter hydrossis

Septicity:

Type 021N

***Thiothrix* I and II**

Beggiatoa

Type 0961

Type 0581

Type 0411

Type 0092

***Nostocoida limicola* I, II, and III**

Type 0914

Nutrient Deficiency:

Type 021N

***Thiothrix* I and II**

***Nostocoida limicola* III**

Haliscomenobacter hydrossis

5 Filament Growth Environments

A microscopic image showing numerous long, thin, filamentous bacteria. Some filaments are straight, while others are curved or tangled. The background is a light, grainy texture, likely representing the surrounding water or sludge.

- **Filaments grow in under specific conditions**
 - **Low F/M**
 - **Low DO**
 - **Oil and Grease**
 - **Septicity, sulfides**
 - **Nutrient Deficiency (usually industrial treatment)**

A microscopic image showing a complex network of thin, dark, branching structures, possibly fungal hyphae or plant roots, against a light gray background. The structures are interconnected and form a dense, web-like pattern. The text "Why Perform a Microscopic Analysis?" is overlaid in the center in a bold, black, sans-serif font.

Why Perform a Microscopic Analysis?



Questions?

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Protozoa and Metazoa

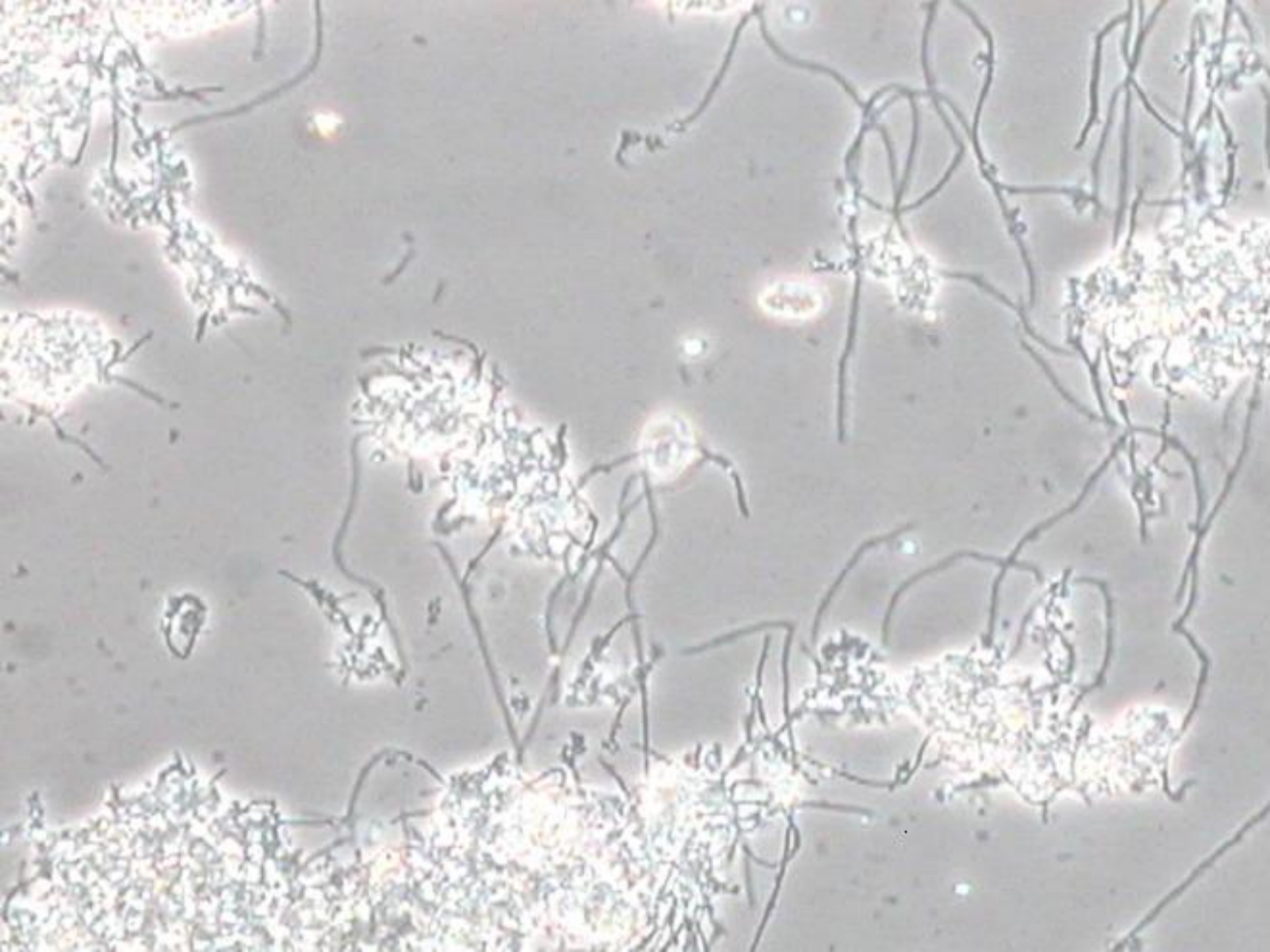


- **Protozoa**

- **Single Cell Animals**
- **Indicator Organisms**
 - **Treatment Levels**
 - **Early Warning System**

- **Metazoa**

- **Multicelled Animals**
- **Old Sludge (waste!)**





***Wastewater Microbiology:
Flocs and Filaments***

Delaware County Sewer District

Wastewater Microbiology Workshop

**Olentangy Environmental Control Center
Delaware, Ohio**

January 12, 2012

Ohio EPA Compliance Assistance Unit

A microscopic image showing a complex network of thin, dark, branching structures, possibly fungal hyphae or plant roots, against a light gray background. The structures are interconnected and form a dense, web-like pattern. The text "Why Stain the Bugs?" is overlaid in the center in a bold, black, sans-serif font.

Why Stain the Bugs?

Filaments Identifiable by Staining

Gram Positive

Microthrix parvicella

Nocardia

Nostocoida limicola

Type 0041/0675

Type 1851

Type 0914

Neisser Positive

Microthrix parvicella (granules)

Nocardia (granules)

Nostocoida limicola

Type 0092

Tools for Staining




- **Microscope**

- **Bright field (not phase contrast!)**
- **Preferably 1000x oil immersion objective**
- **Minimum of 200x objective**

Tools for Staining

- **Microscope Slides**
- **Clothes pin**
- **Wash bottle**
- **Watch with a second hand**
- **Paper towel**
- **Bon Ami Scouring Powder (optional)**

Tools for Staining

The background of the slide is a microscopic image of a Gram-stained bacterial smear. It shows numerous purple-stained cocci (Gram-positive) and pink-stained cocci (Gram-negative) arranged in various patterns, including chains and clusters. The purple-stained bacteria are more numerous and appear as small, dark purple dots, while the pink-stained bacteria are fewer and appear as small, light pink dots. The overall appearance is that of a mixed bacterial culture.

- **Gram Stain Kit**
 - **Gram Crystal Violet Solution**
 - **Gram Iodine Solution**
 - **Gram Decolorizing Solution**
 - **Gram Safranin Solution**

Gram Staining Procedure

1. Gram Crystal Violet Solution
 - Flood slide for 1 minute
 - Rinse with DI water
2. Gram Iodine Solution
 - Flood slide for 1 minute
 - Rinse with DI water
3. Gram Decolorizing Solution
 - Hold slide at 45 degrees and apply dropwise until blue color stops rinsing off
 - Blast with DI water to stop reaction, blot dry with paper towel
4. Gram Safranin Solution
 - Flood slide for 1 minute
 - Rinse with DI water
5. View Slide at 1000x under bright light (not phase contrast)

Tools for Staining

- **Neisser Stain Kit**
 - **Neisser Methyl Blue Solution A**
 - **Neisser Crystal Violet Solution B**
 - **Neisser Bismark Brown Solution**
 - **Transfer pipet**
 - **Container for mixing Solutions A and B**

Neisser Staining Procedure

1. Methyl Blue / Crystal Violet Solution

- Mix 2 parts Methyl Blue and 1 part Crystal Violet in a small container
- Flood slide for 30 seconds
- Rinse with DI water

2. Bismark Brown Solution

- Flood slide for 1 minute
- Rinse with DI water and blot dry (do not rub the slide)

3. View Slide at 1000x bright light (not phase contrast)



Questions?

Contact:

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