

A microscopic view of wastewater treatment tank sludge, showing a dense network of fine, thread-like structures and various small, irregular particles, likely representing microbial communities.

# **The Microbiology of Wastewater Treatment**

## **Life in the Aeration Tank: Bacteria, Protozoa, and Metazoa**

### **USEPA Webinar Series**

**November 17, 2020**

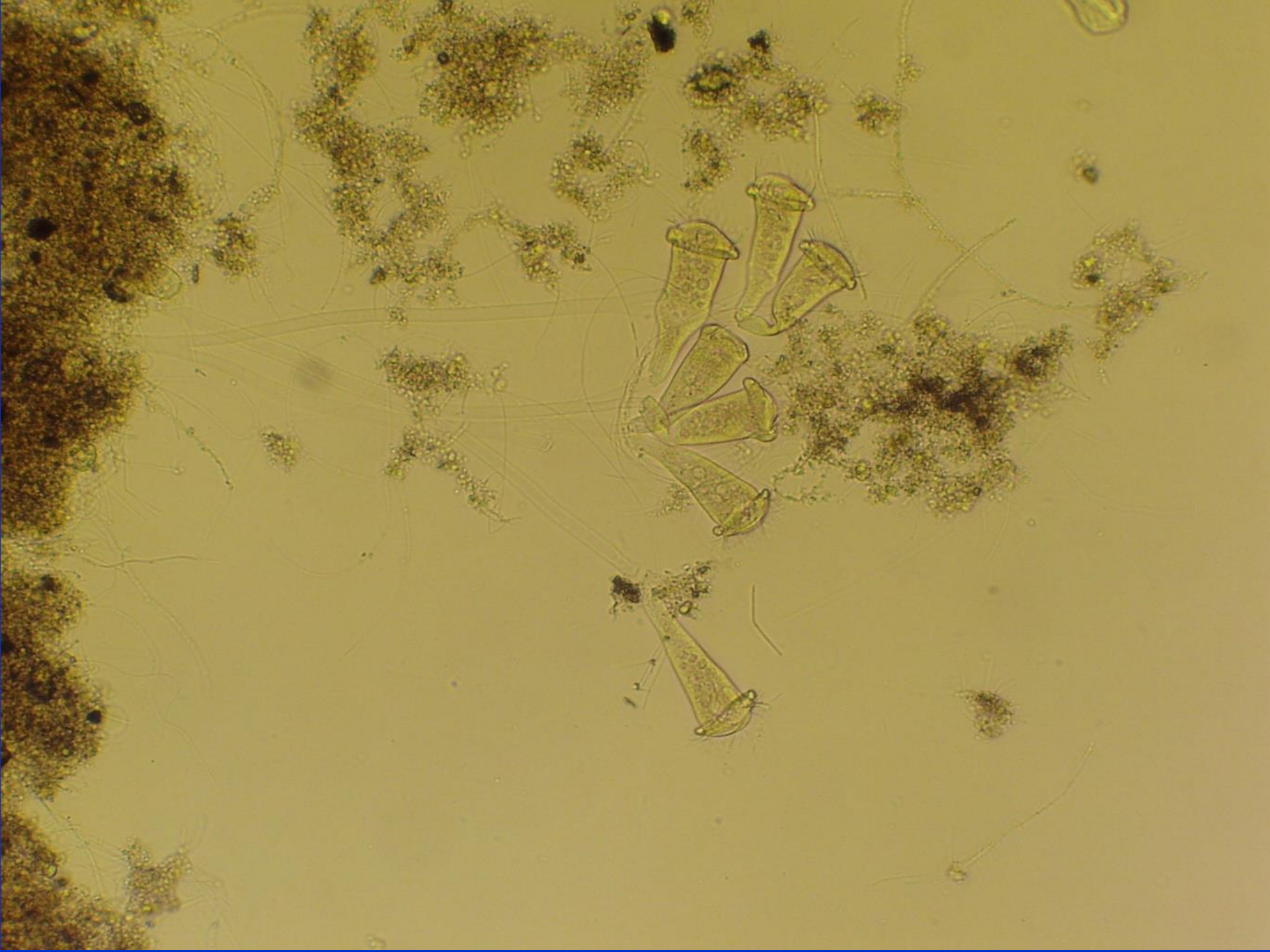
*Jon van Dommelen*

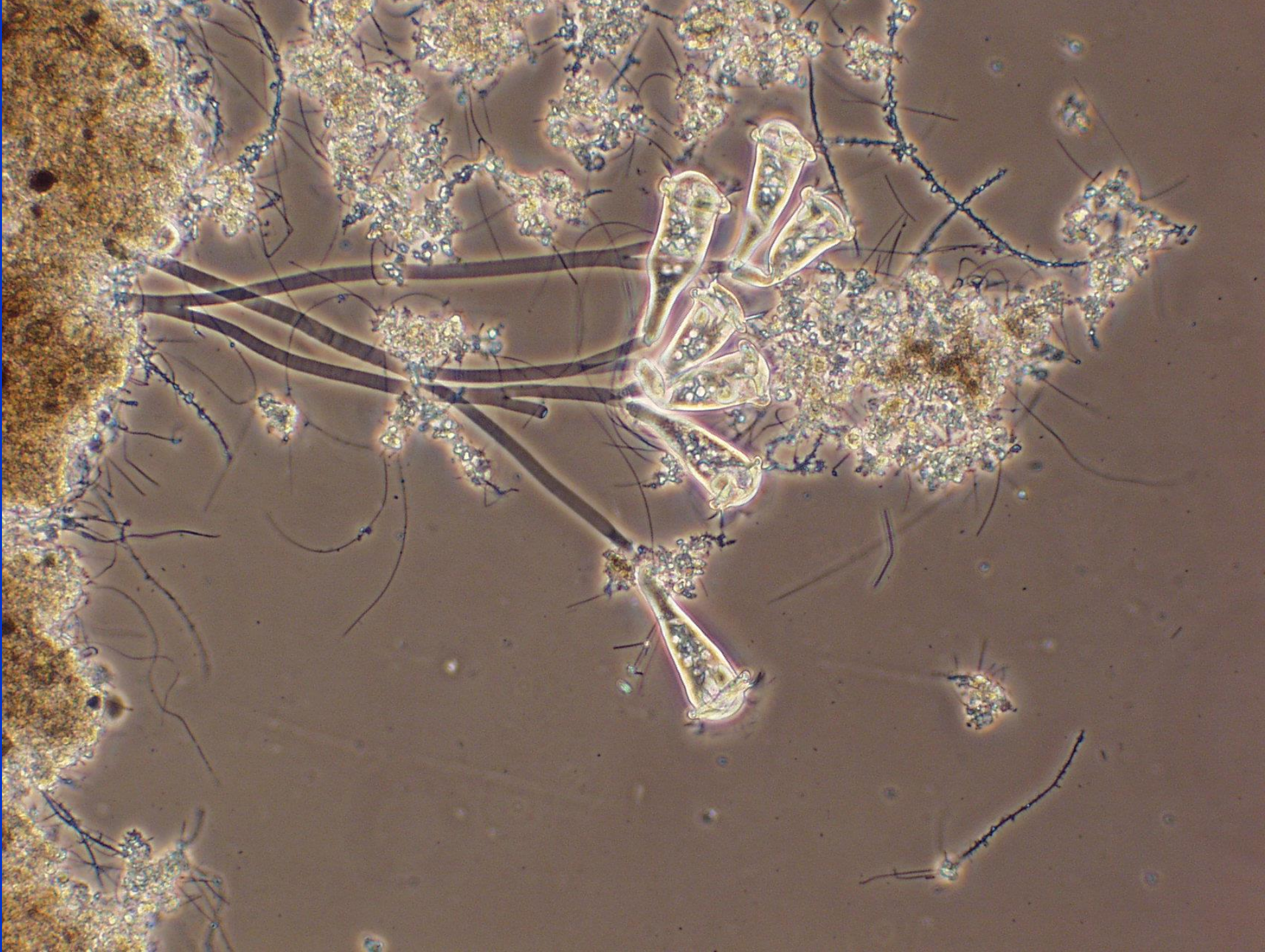
*Ohio EPA Compliance Assistance Unit*

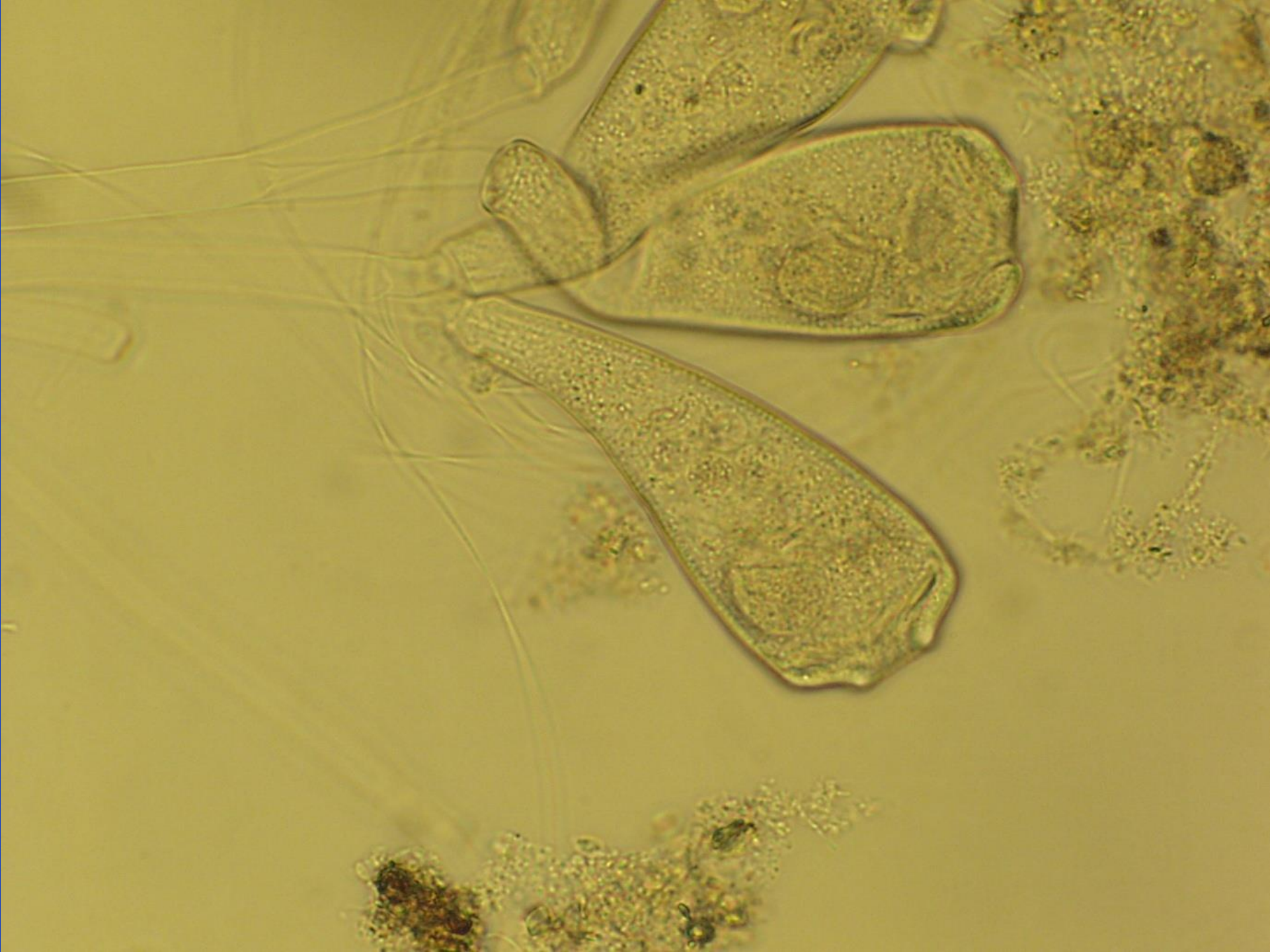
A microscopic image of plant tissue, likely a leaf cross-section, showing various cells and structures. The image is overlaid with a large, bold, black text box. The text reads: "Why Does My Microscopic Work Better than Yours?". The background shows a network of thin, dark lines representing cell walls, with some larger, more complex structures that could be stomata or other specialized cells. The overall appearance is that of a typical light micrograph of a plant specimen.

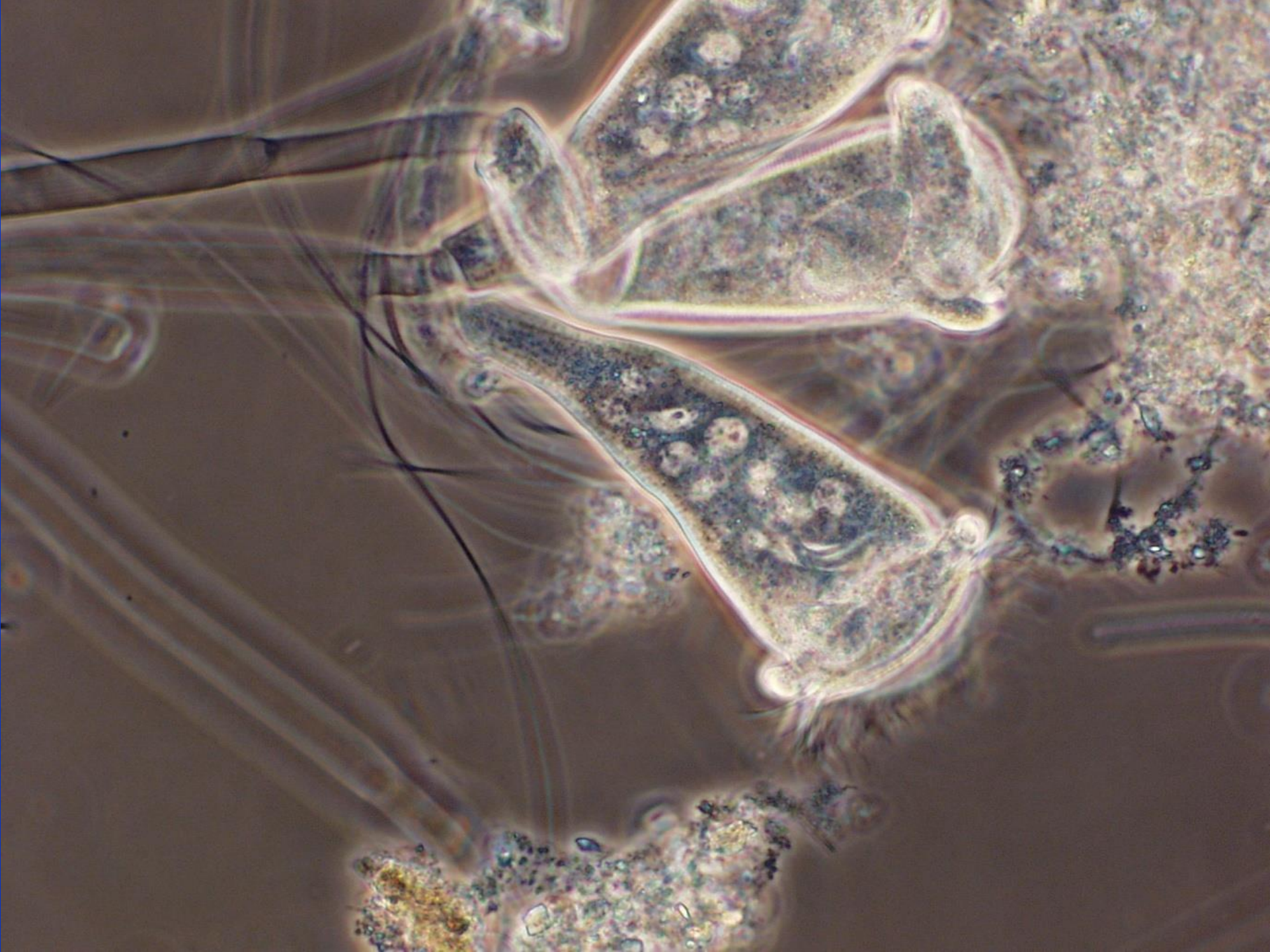
**Why Does My Microscopic  
Work Better than Yours?**







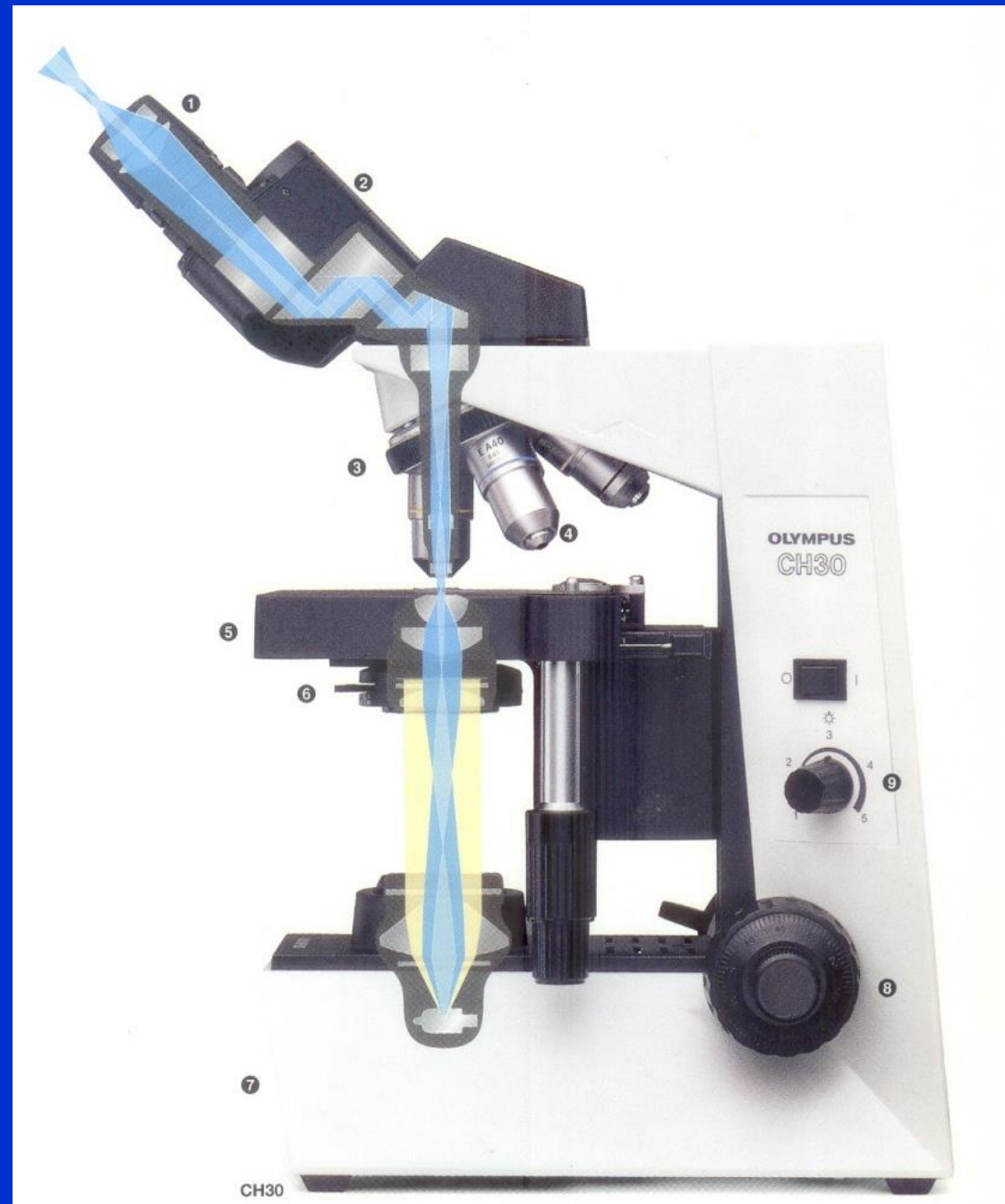




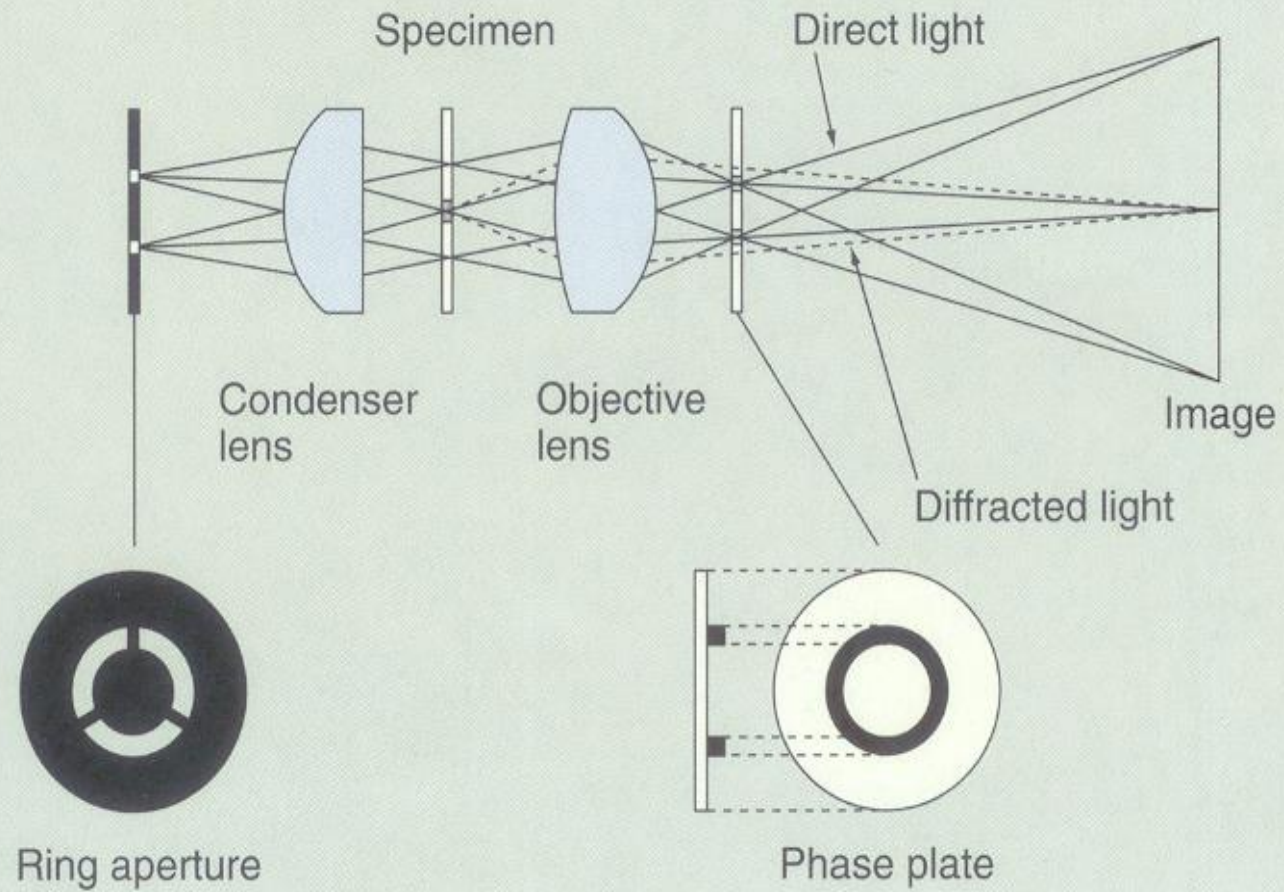








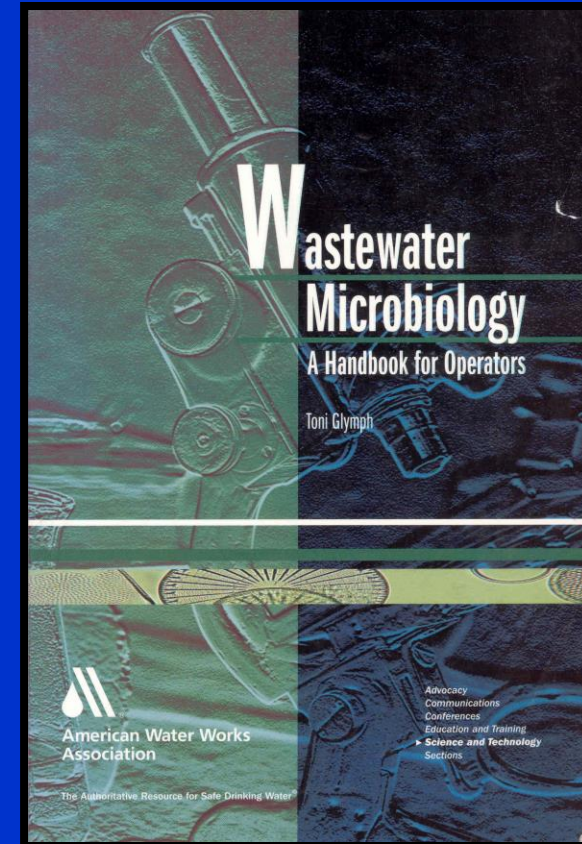
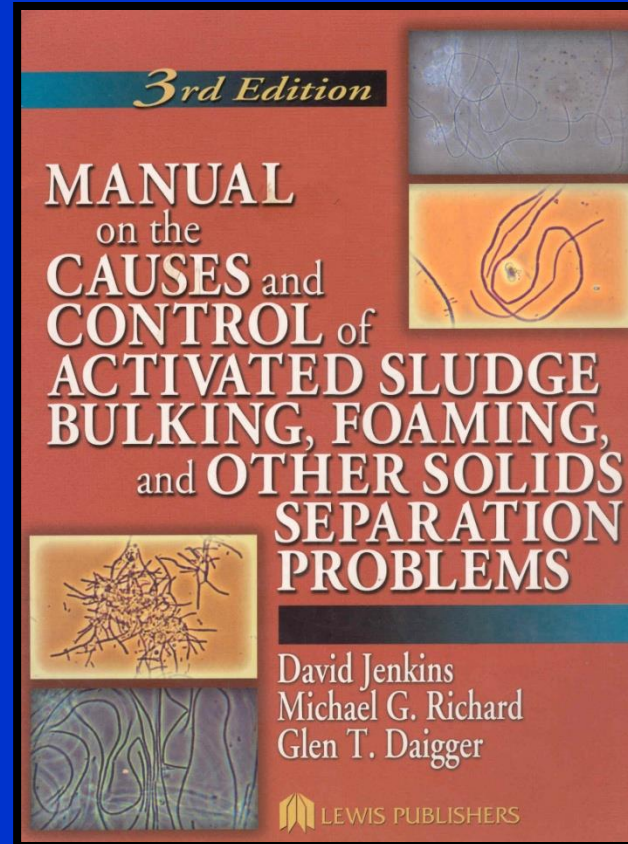
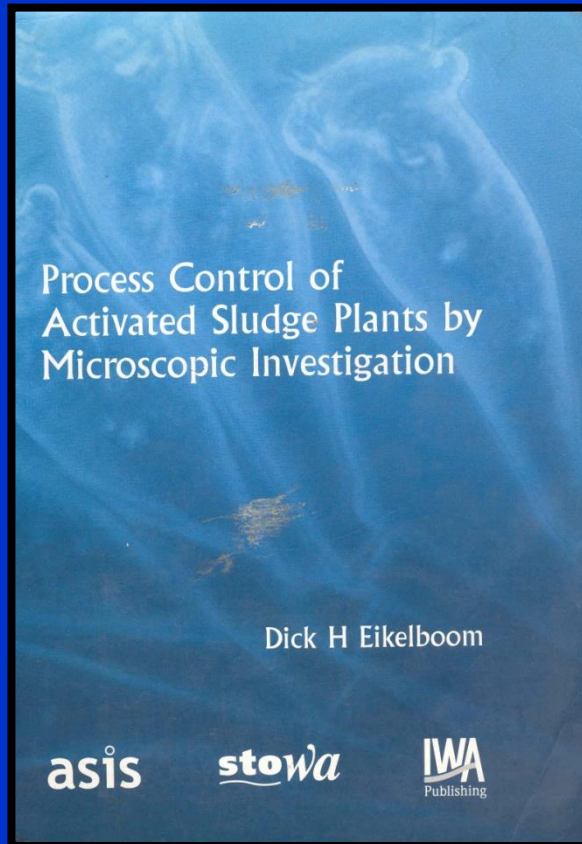
**Fig. 1. Phase Contrast Microscopy**



A microscopic image of plant tissue, likely a leaf cross-section, showing various cellular structures. The image is overlaid with the text "Why Perform a Microscopic Analysis?". The background shows a network of thin, dark lines representing cell walls, with some larger, more complex structures that could be stomata or other specialized cells. The overall appearance is that of a detailed biological specimen under a microscope.

**Why Perform a Microscopic Analysis?**

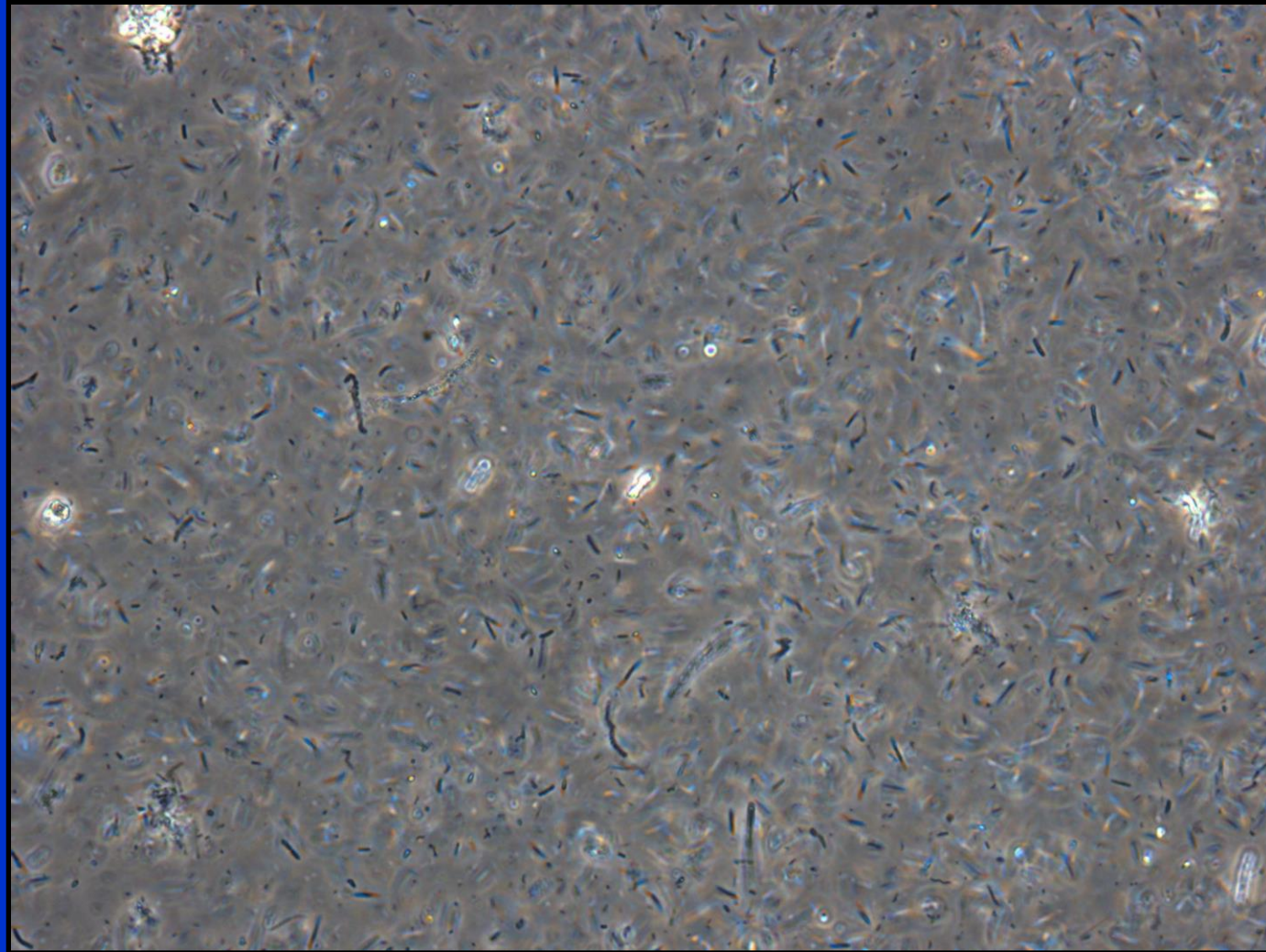
# Essential Resources



A microscopic image showing a complex, interconnected network of fine, dark, thread-like structures. These threads are densely packed in some areas, forming a mesh-like structure, and more sparse in others. The overall appearance is that of a highly branched, fibrous network. The background is a light, uniform color. The text "Floc Structure" is overlaid in the center in a bold, black font.

# Floc Structure

# Dispersed Bacteria

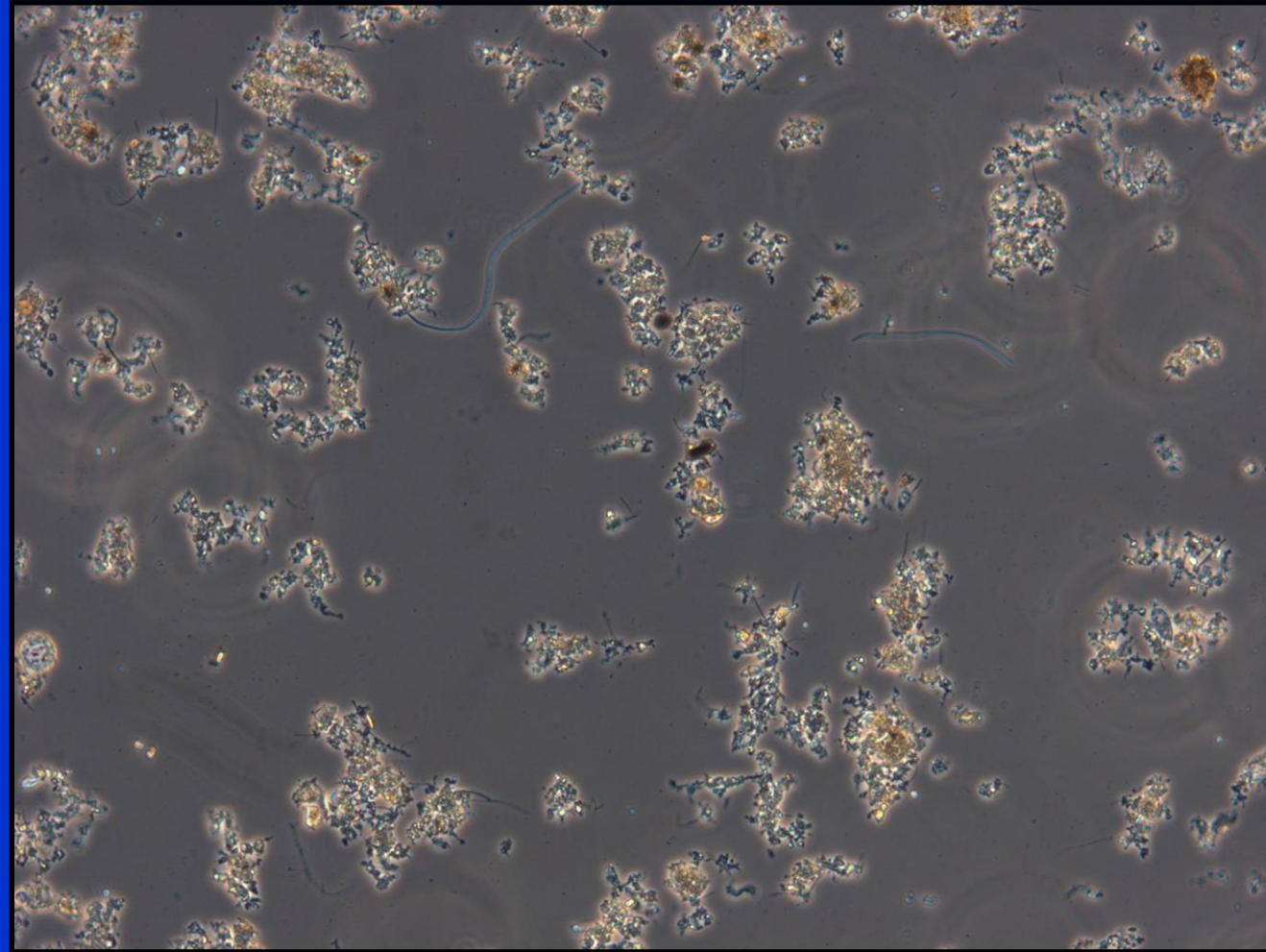


# Beginning of Flocculation

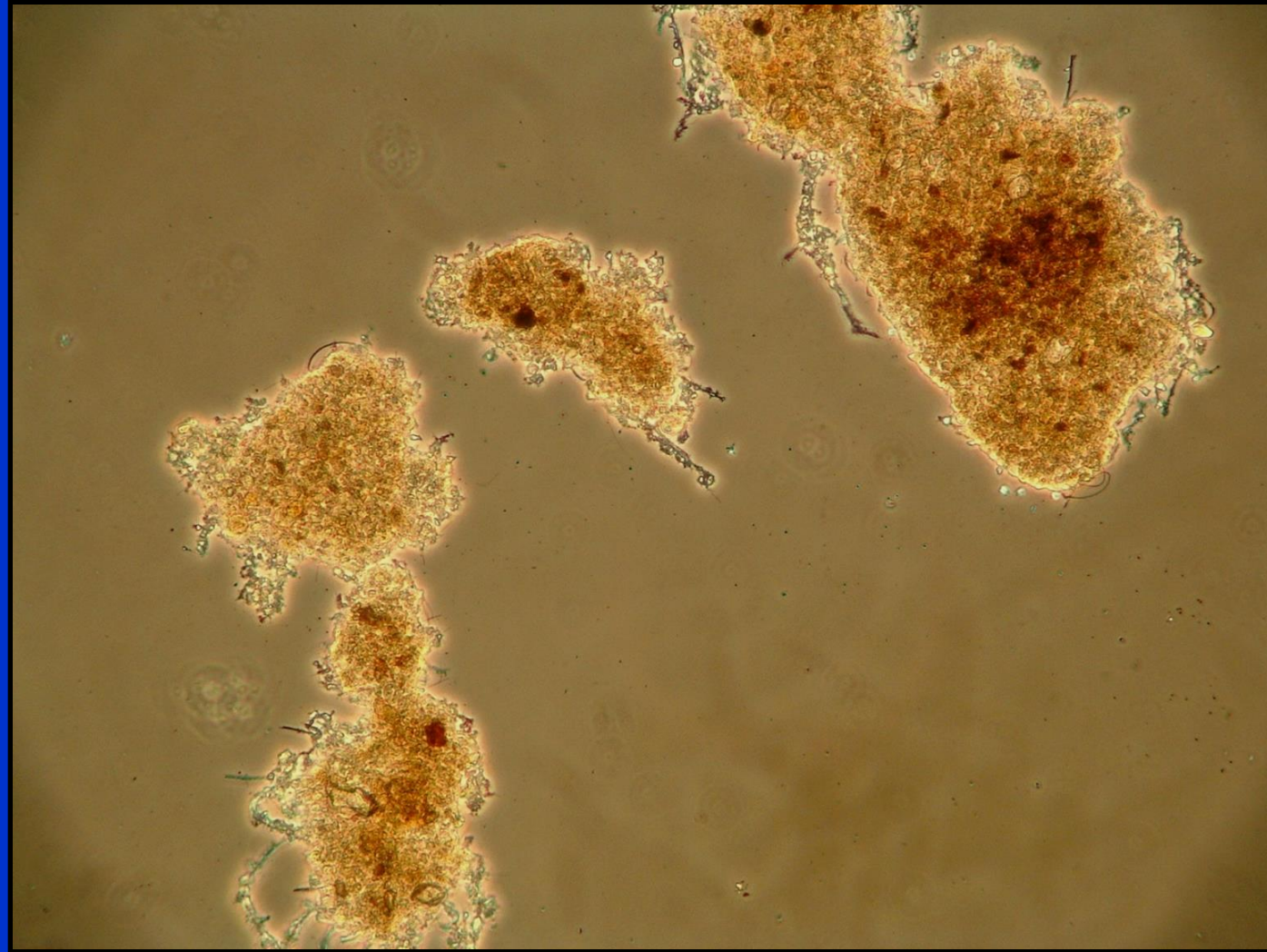




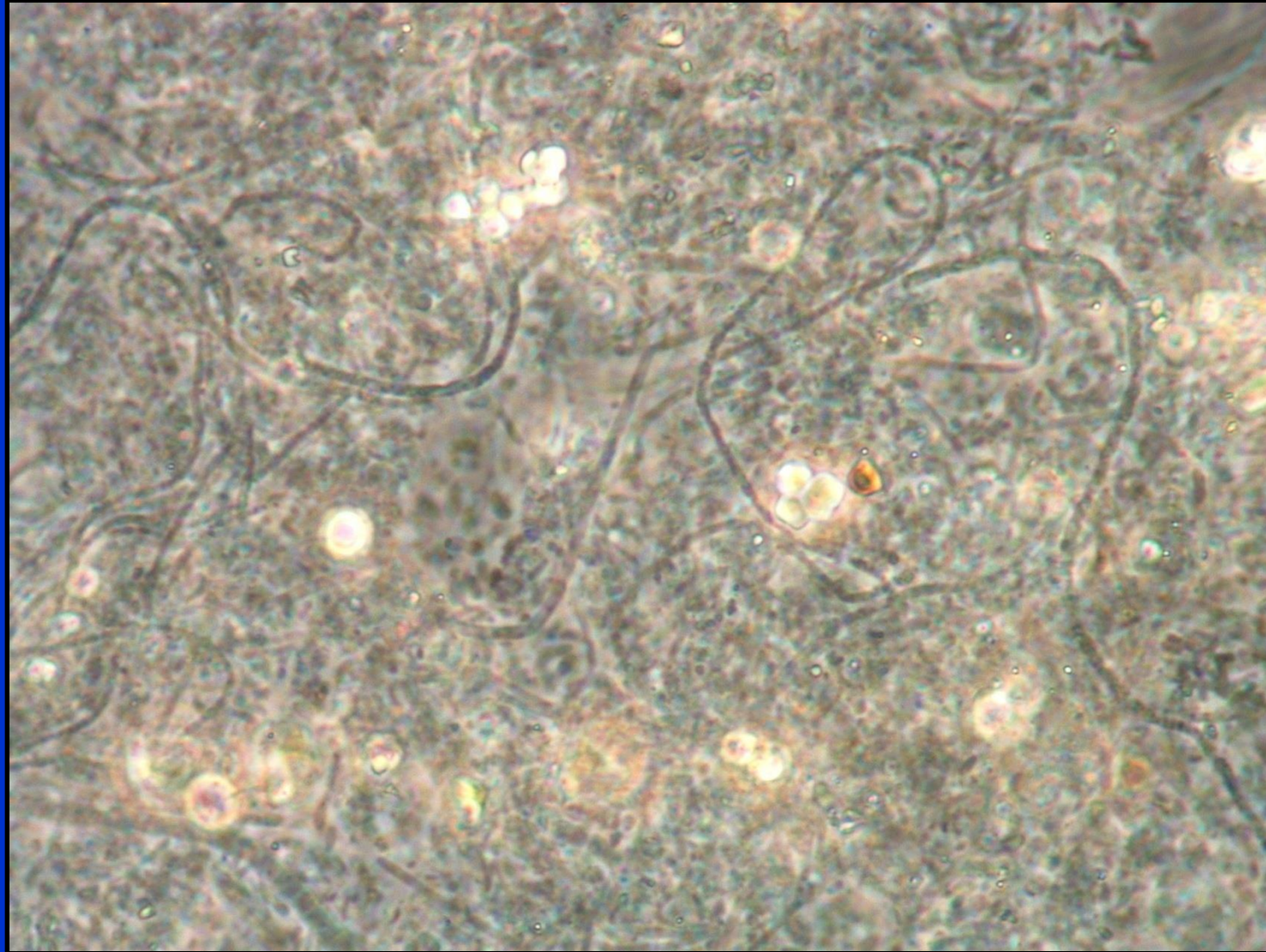
# Small Flocs



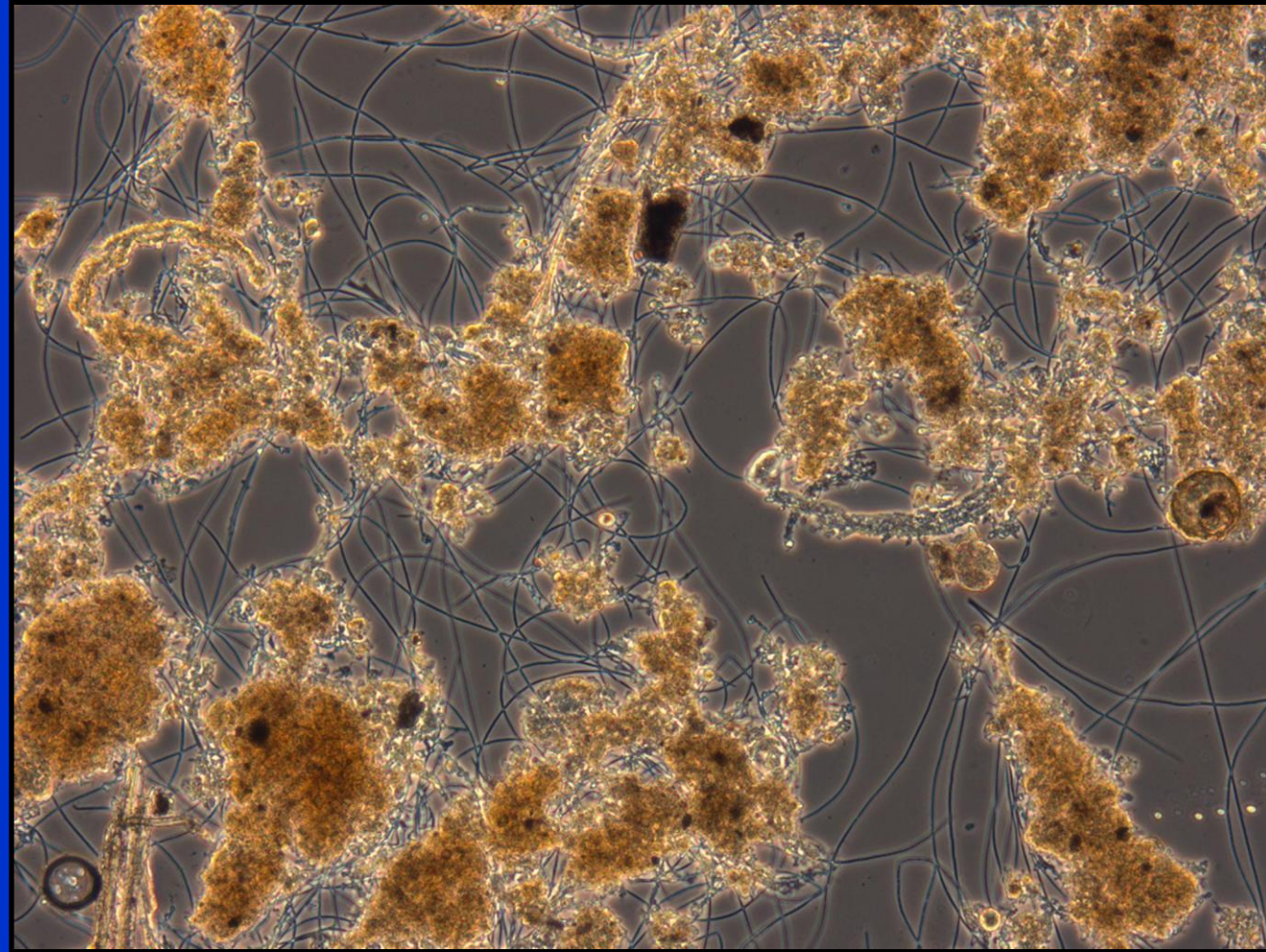
# Dense, Compact Floccs



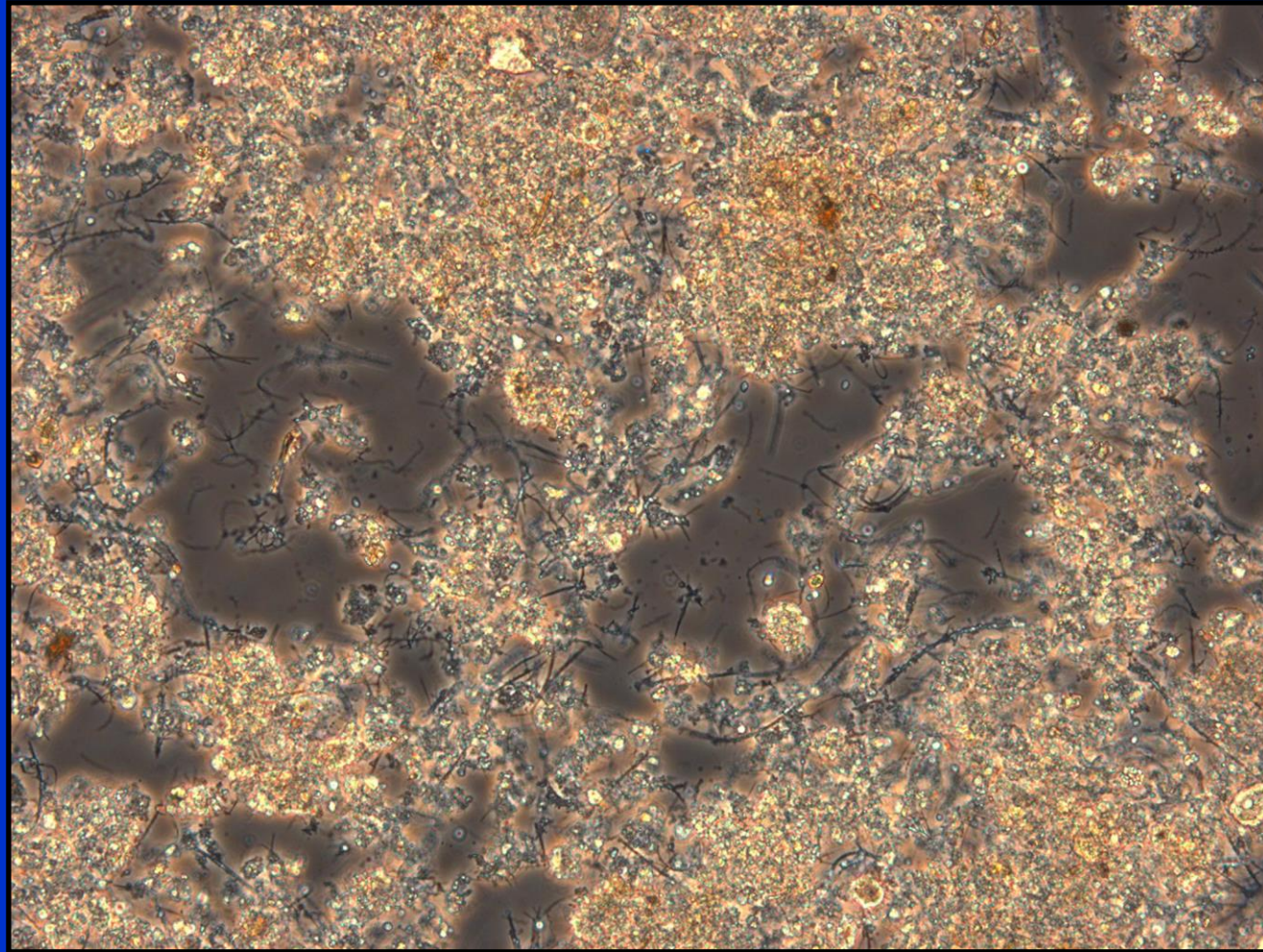
# Internal Filaments Low Density Floccs



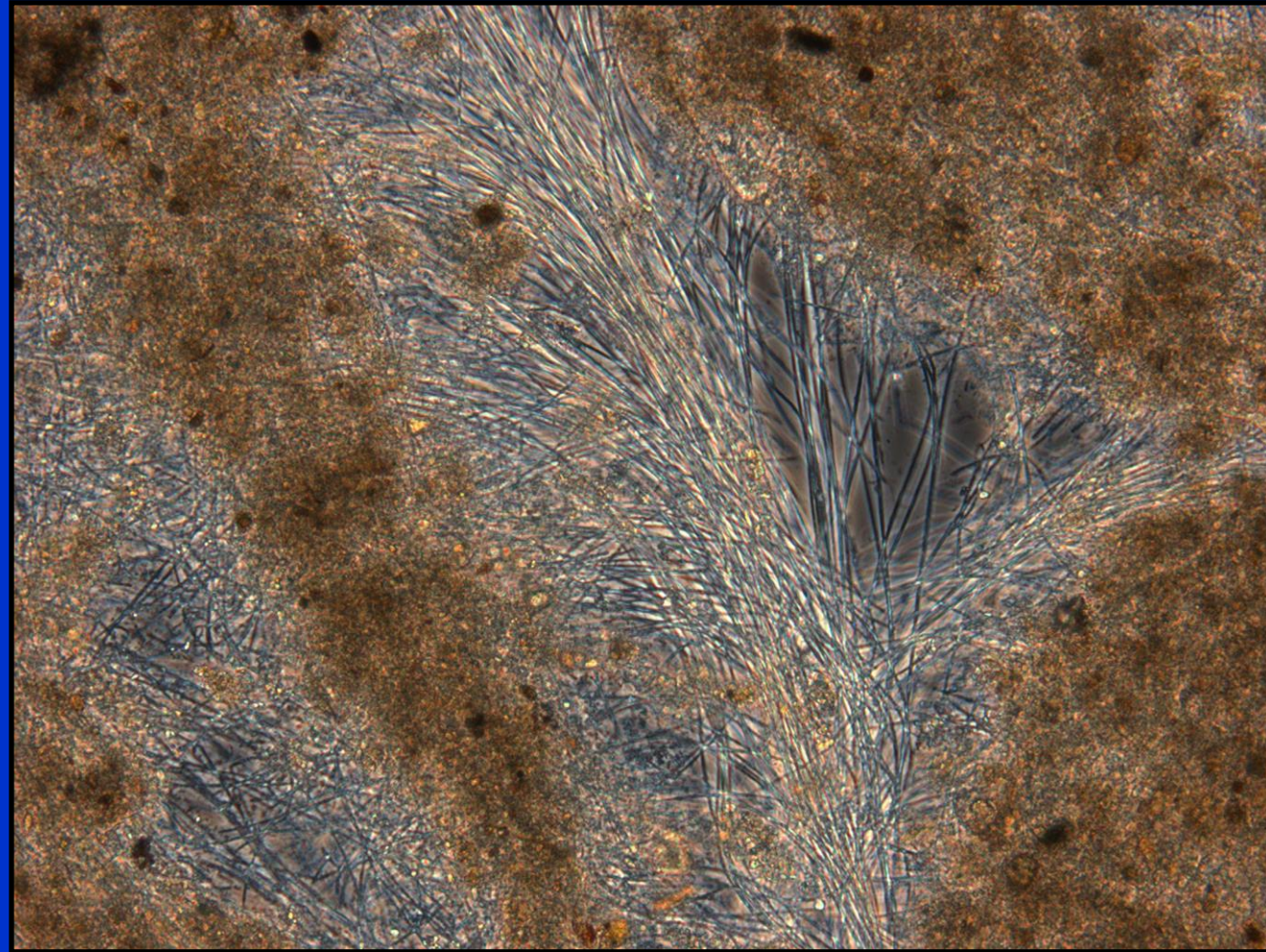
# Extending Filaments Interfloc Bridging

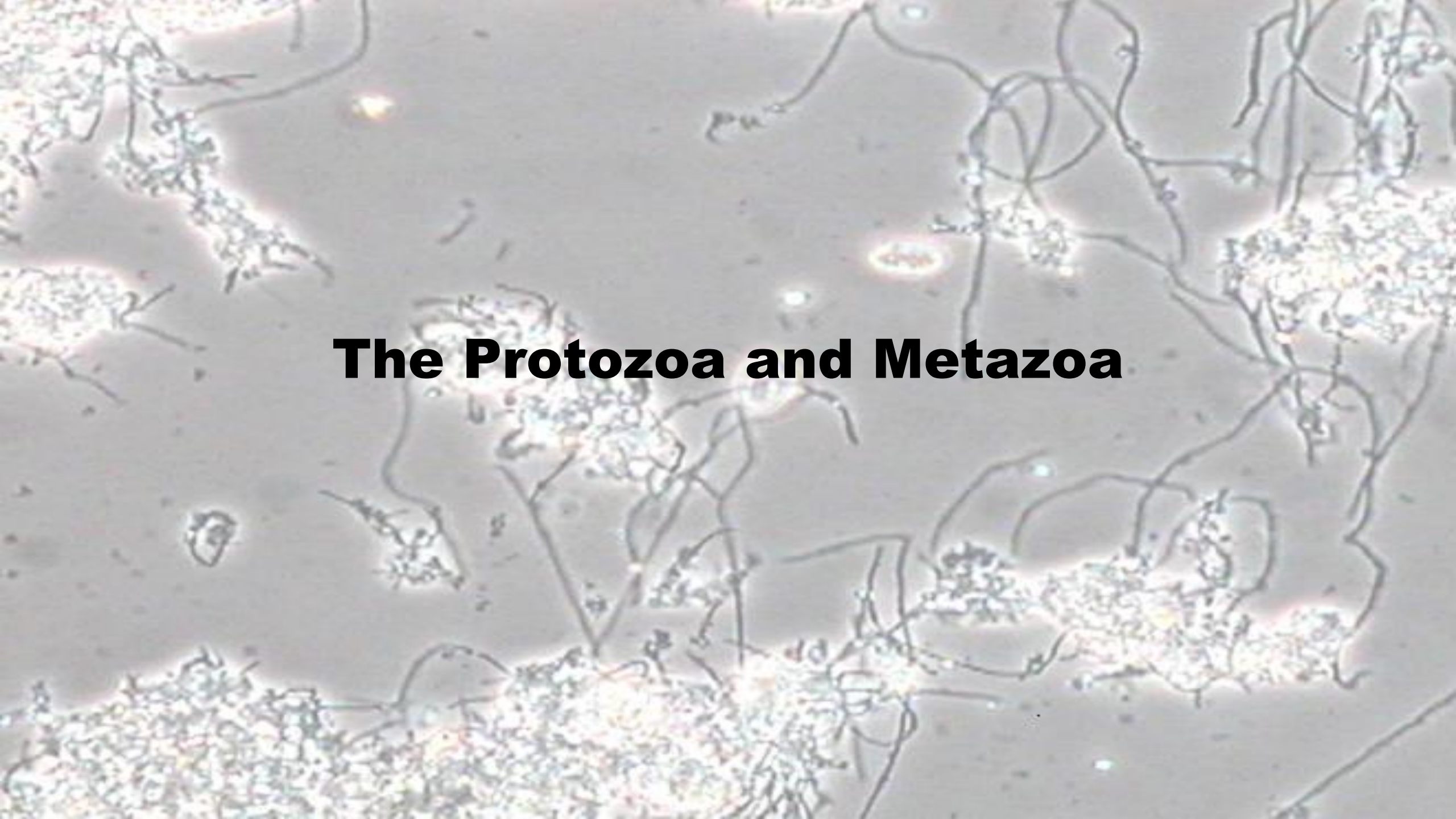


# Too Much Mass



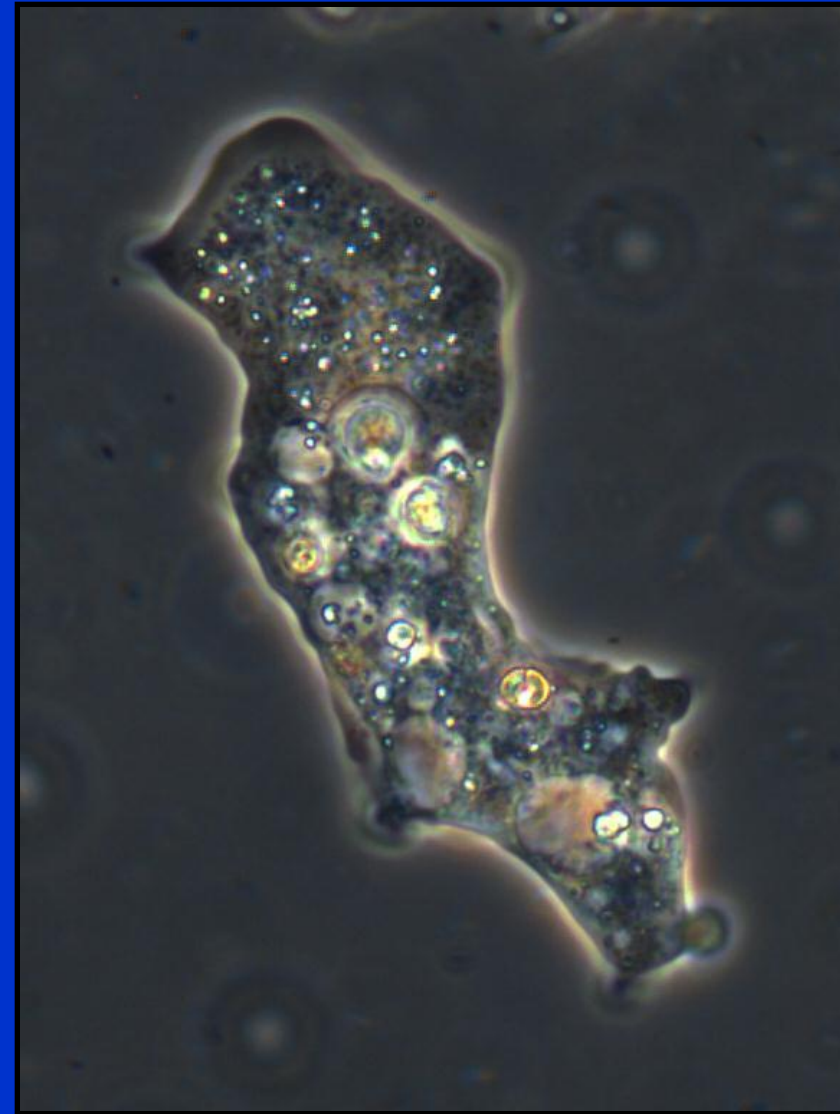
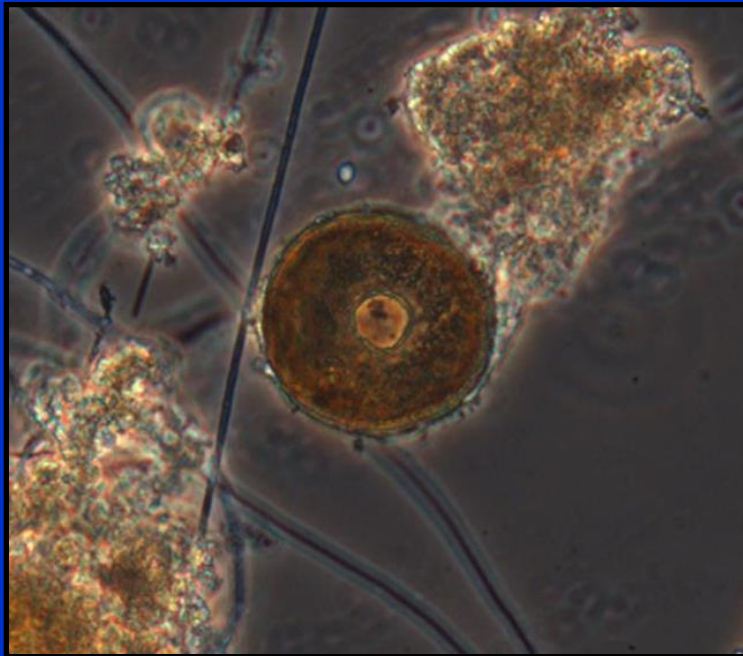
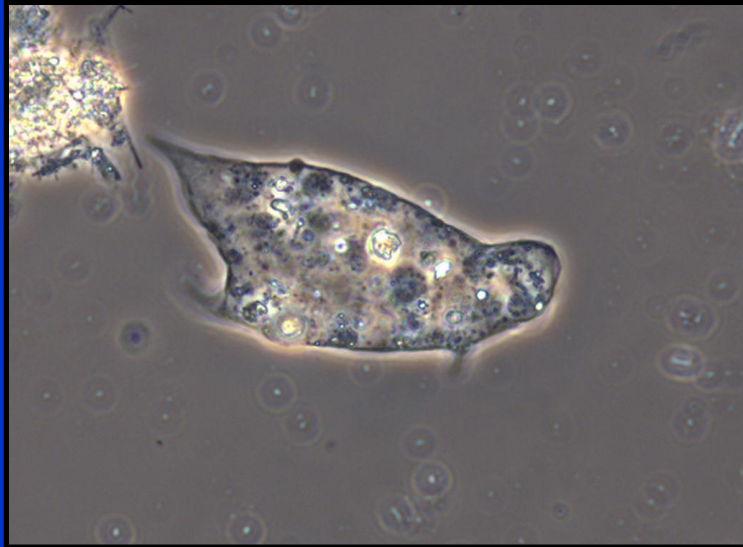
# Too Much Filament



A microscopic image showing a complex network of thin, dark, branching structures, likely biological in origin. The structures are set against a light, slightly textured background. In the center of the image, there is a prominent text overlay. The text is in a bold, black, sans-serif font. The overall appearance is that of a biological specimen, possibly a microorganism or a tissue section, viewed under a microscope.

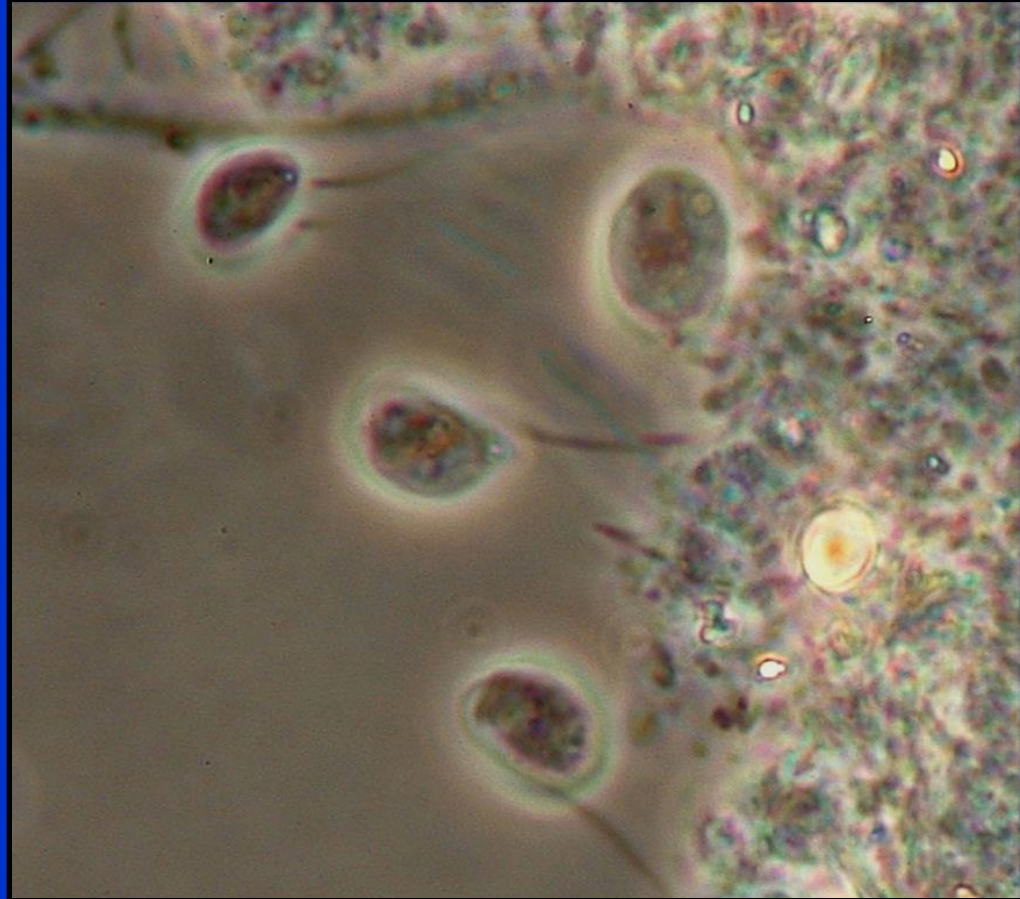
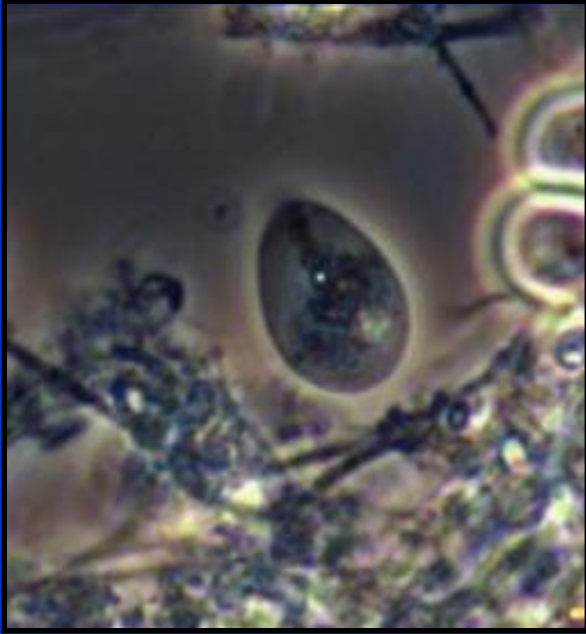
# **The Protozoa and Metazoa**

# Amoeba

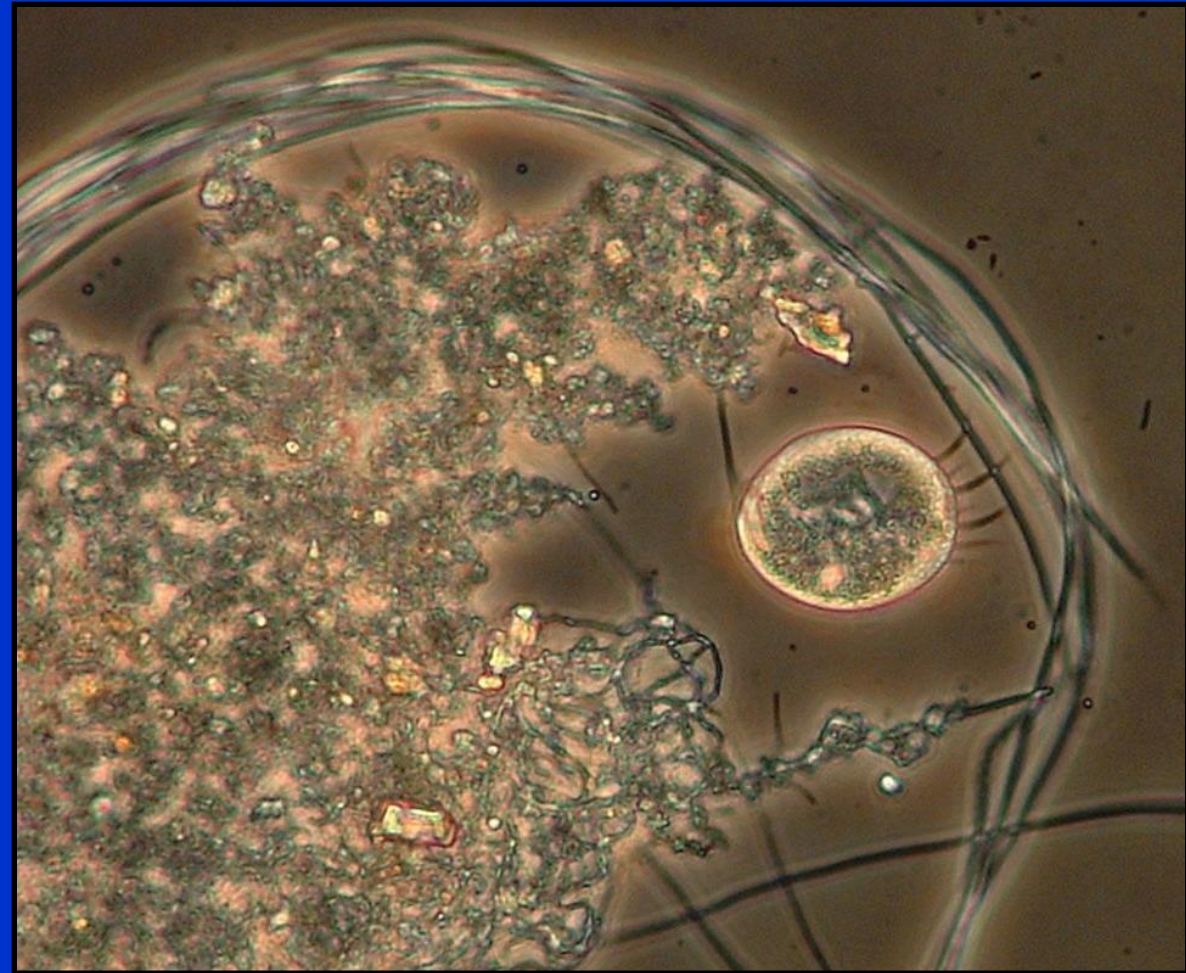
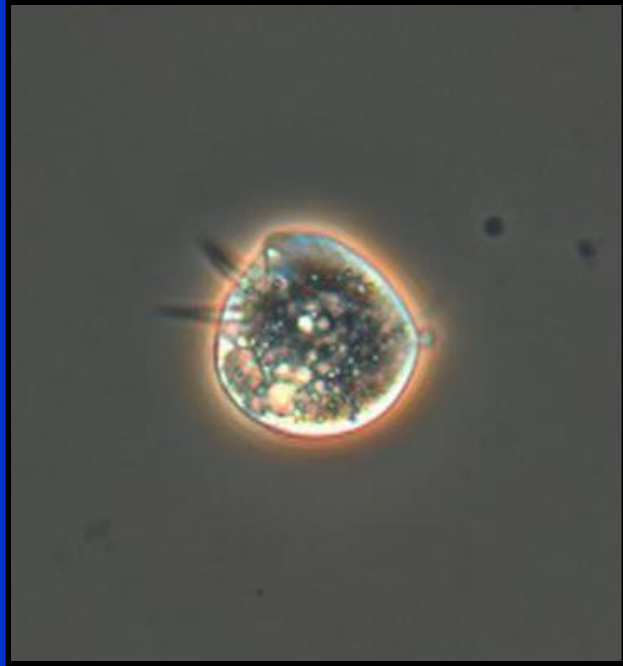




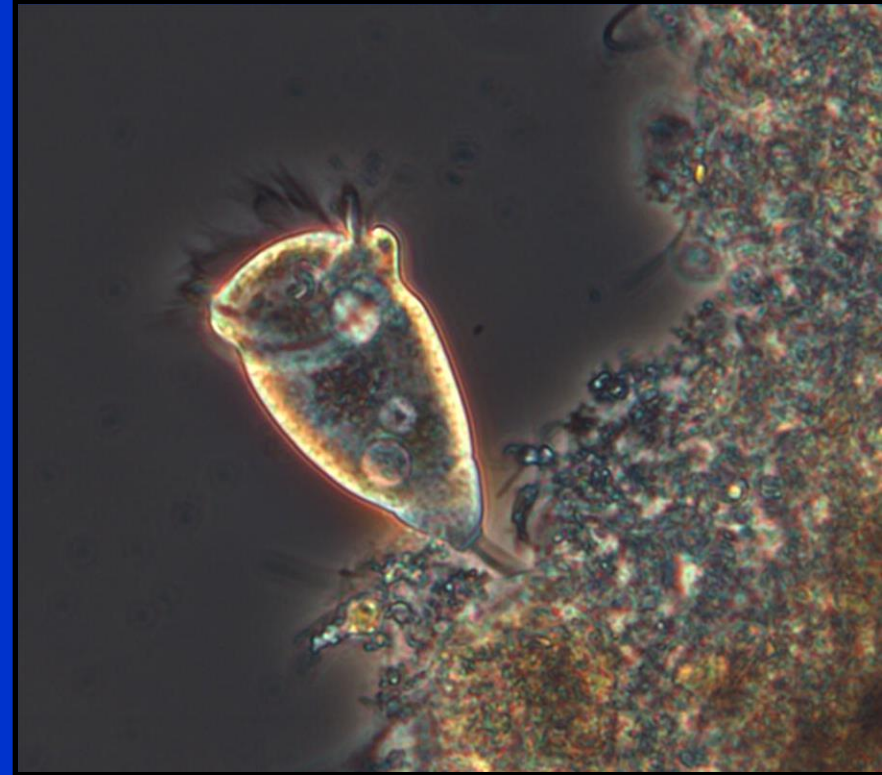
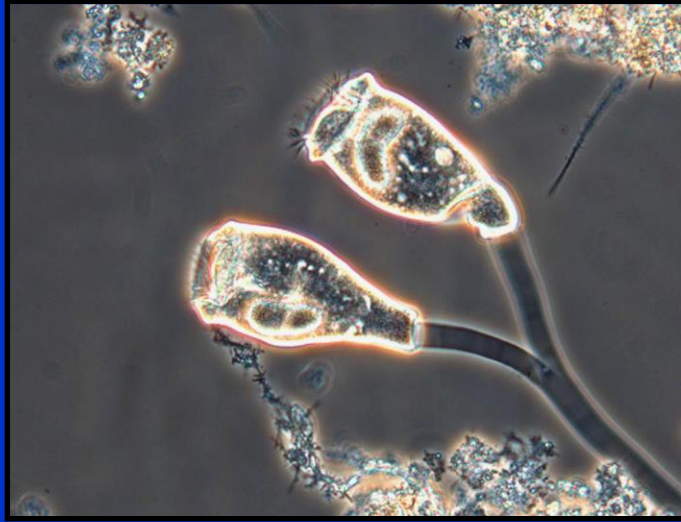
# Flagellates



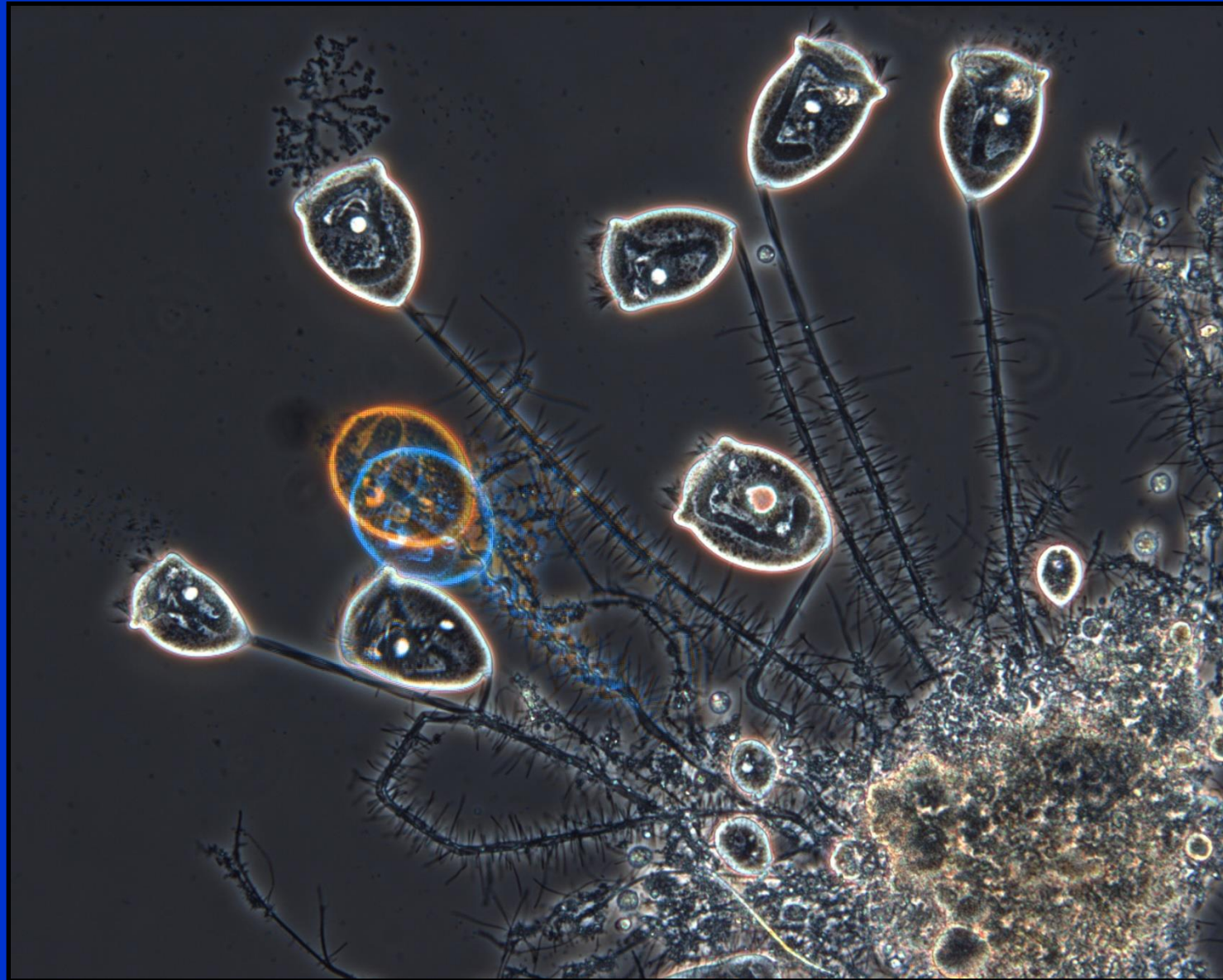
# Crawling Ciliates



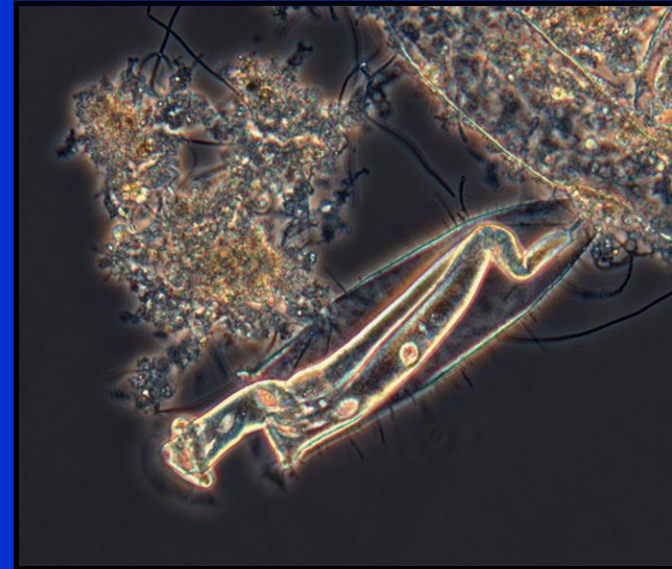
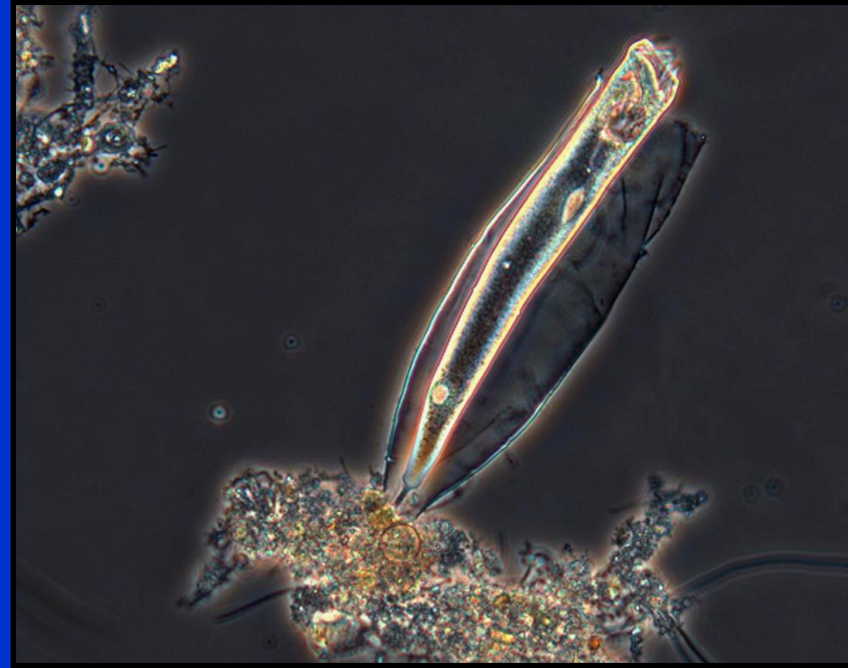
# Stalked Ciliates



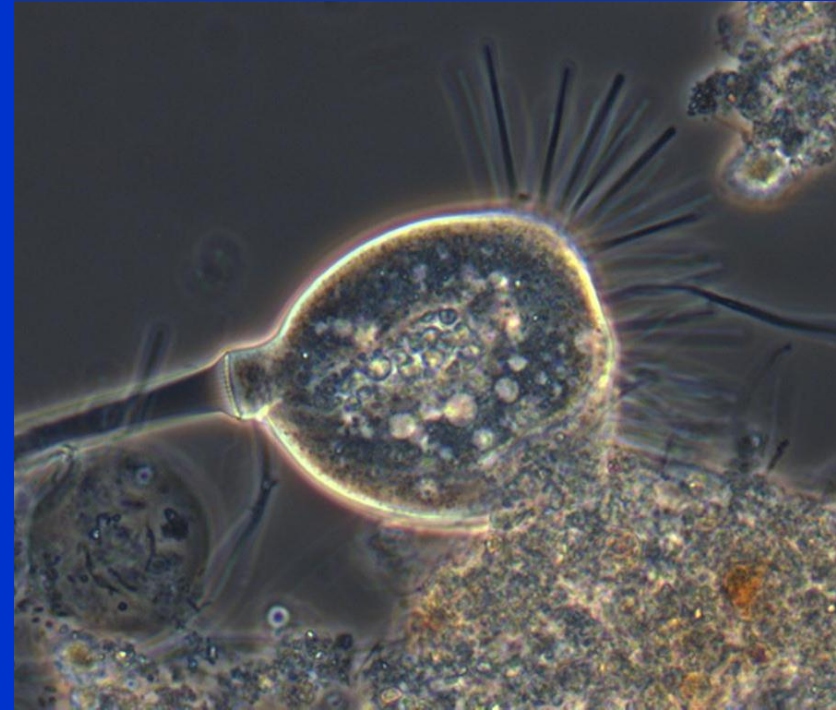
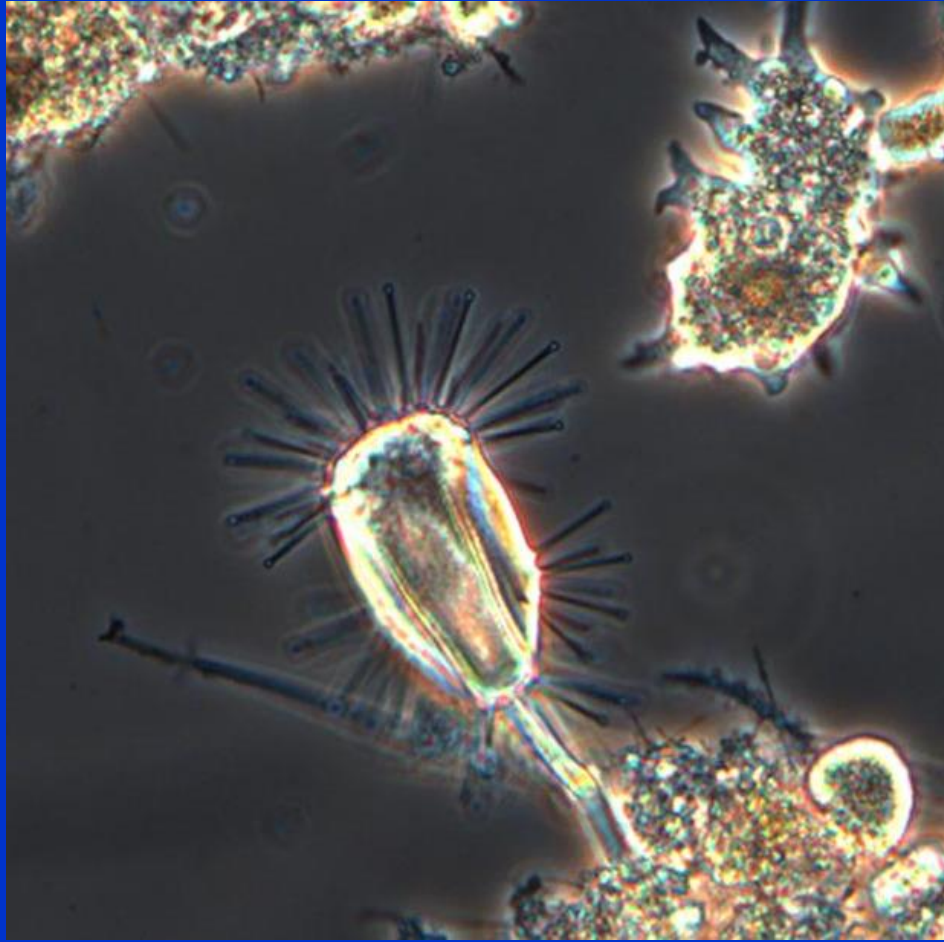
# Stalked Ciliates



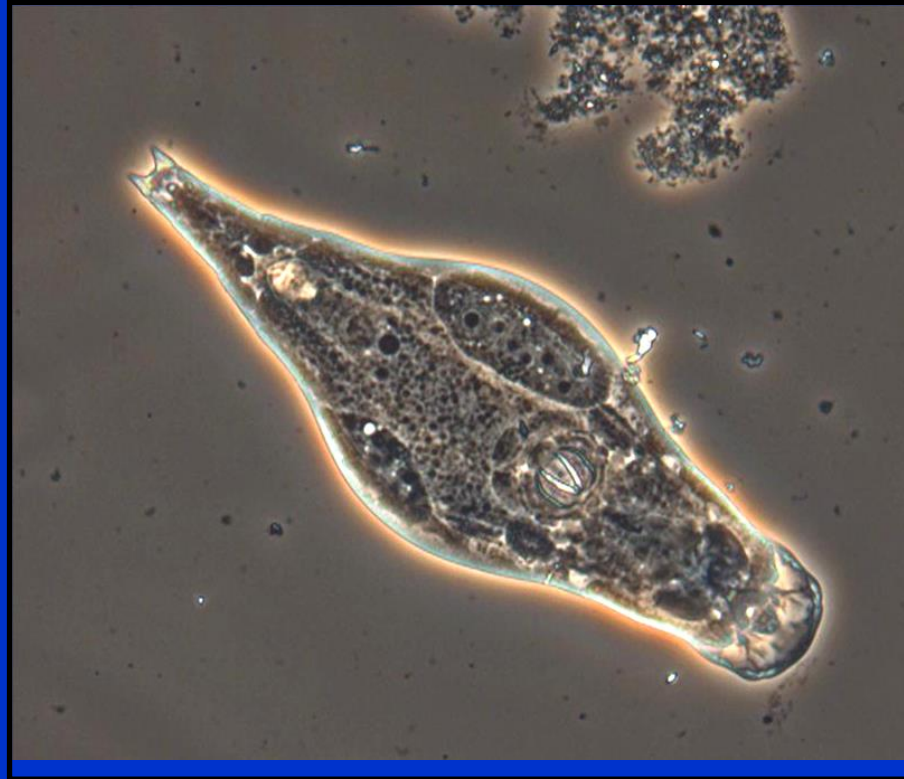
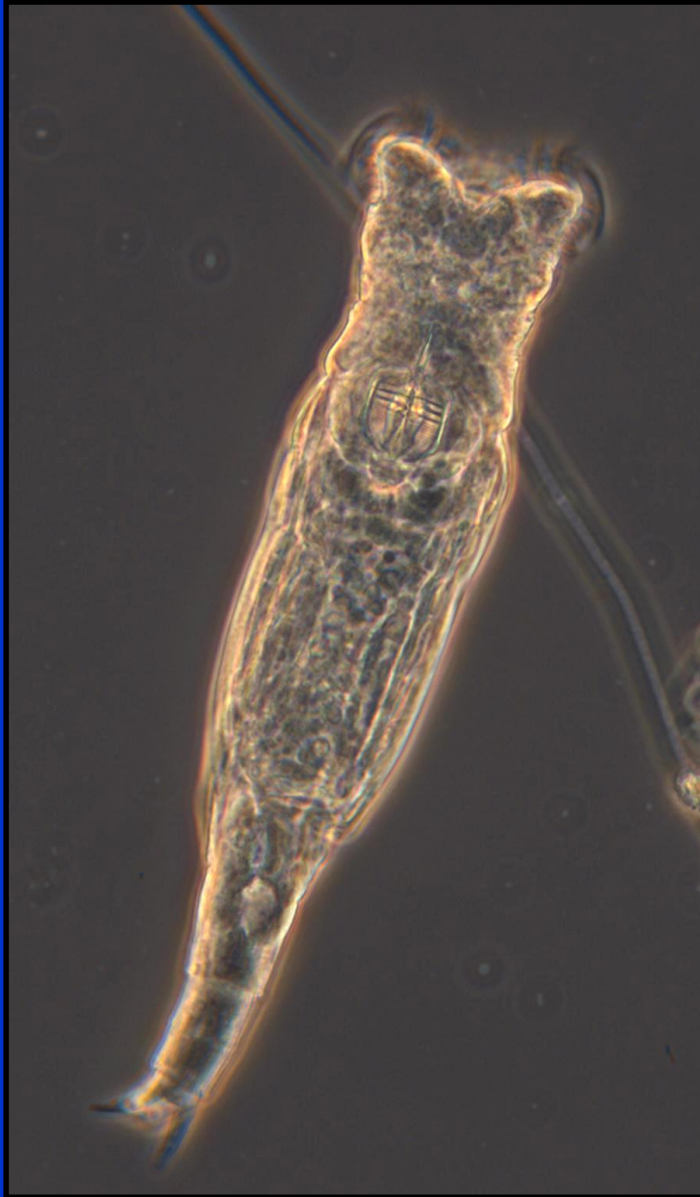
# Vaginicola



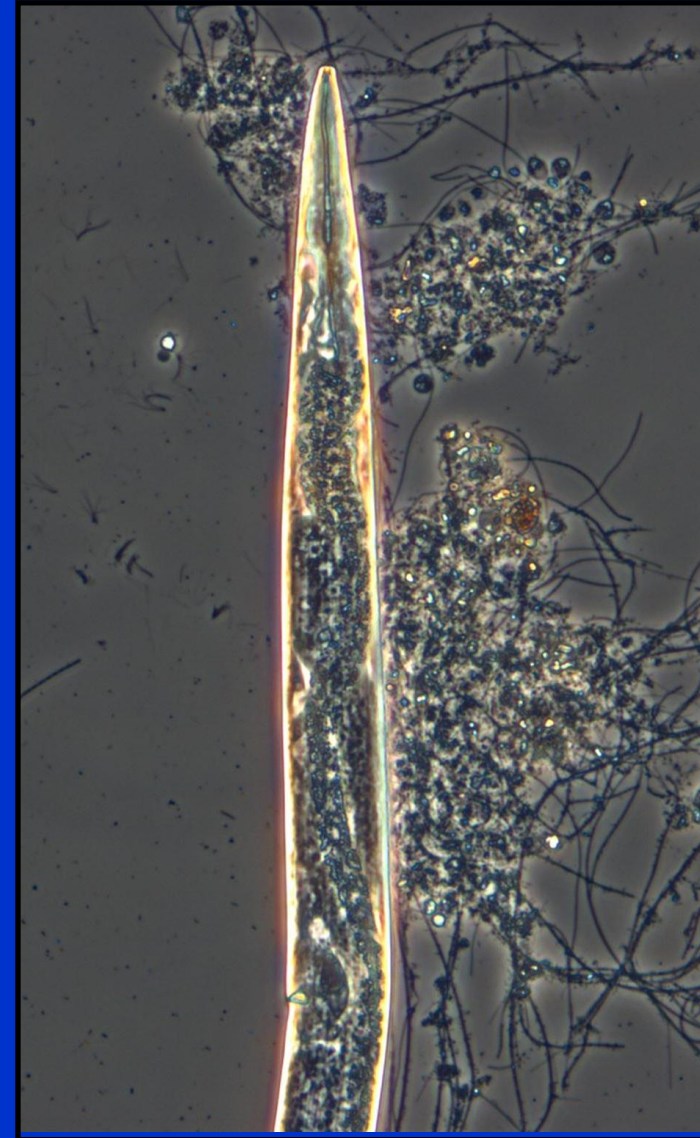
# Suctoria



# Rotifers

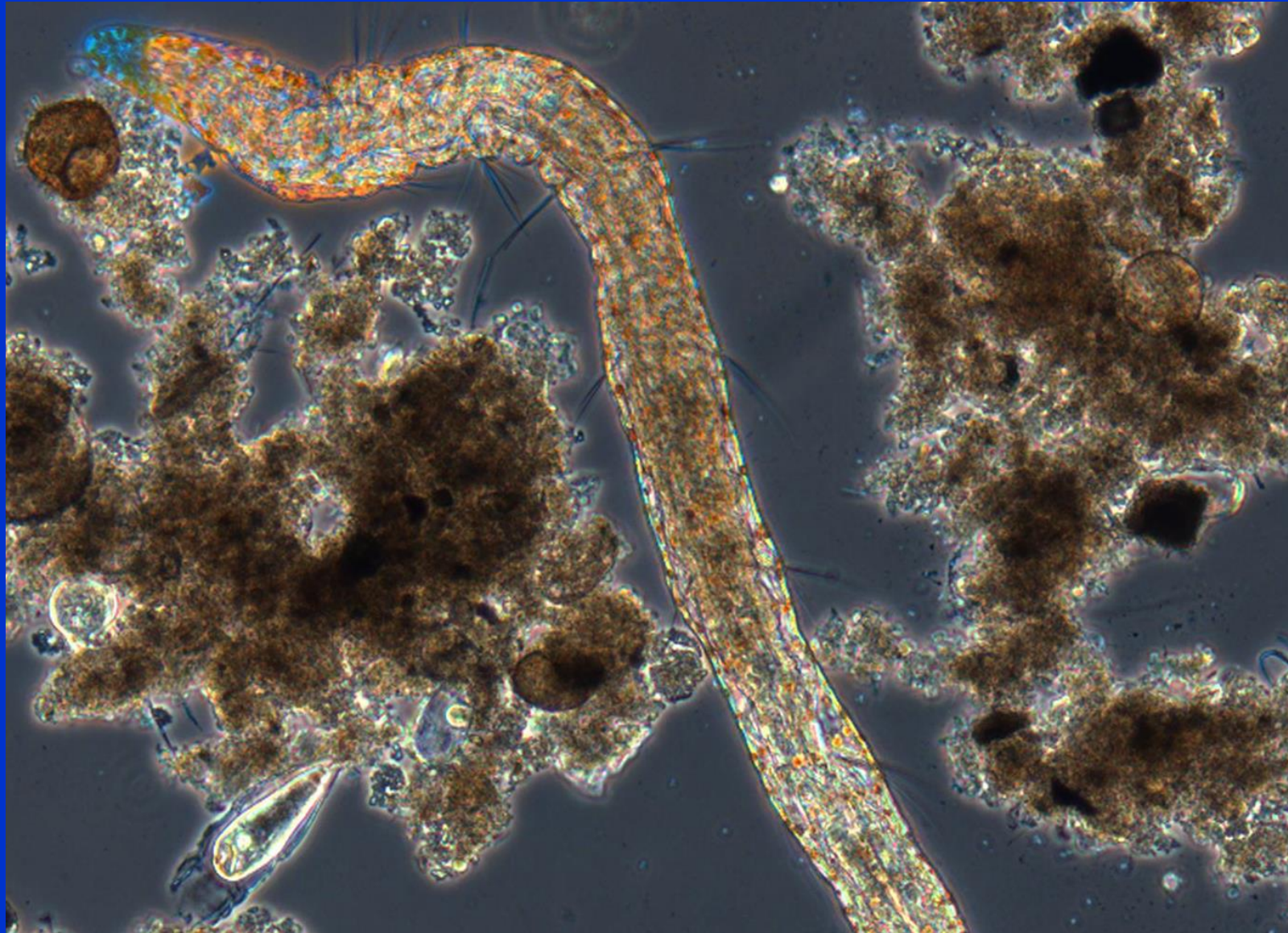


# Nematodes





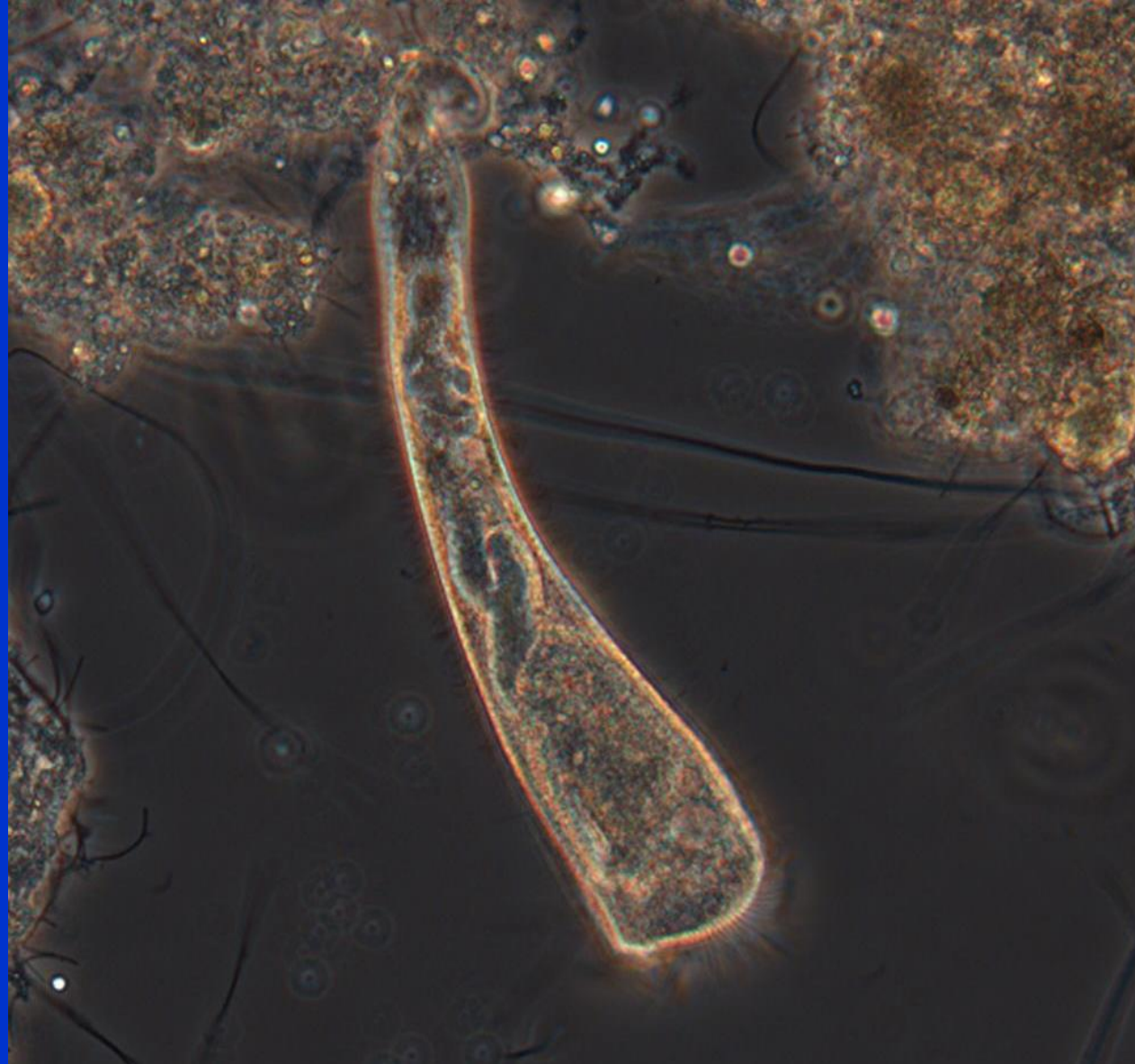
# Bristleworm



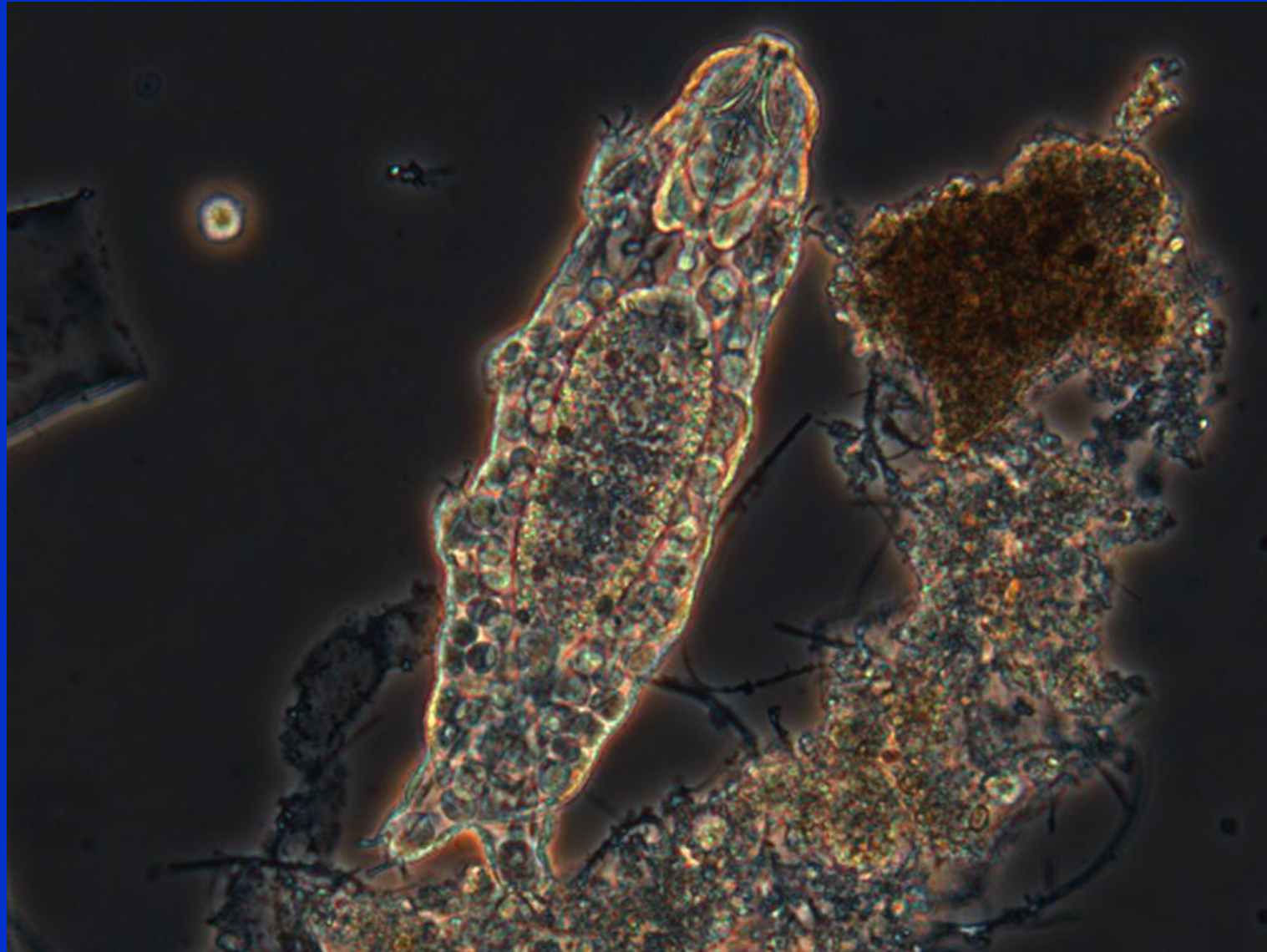
# Paramecium

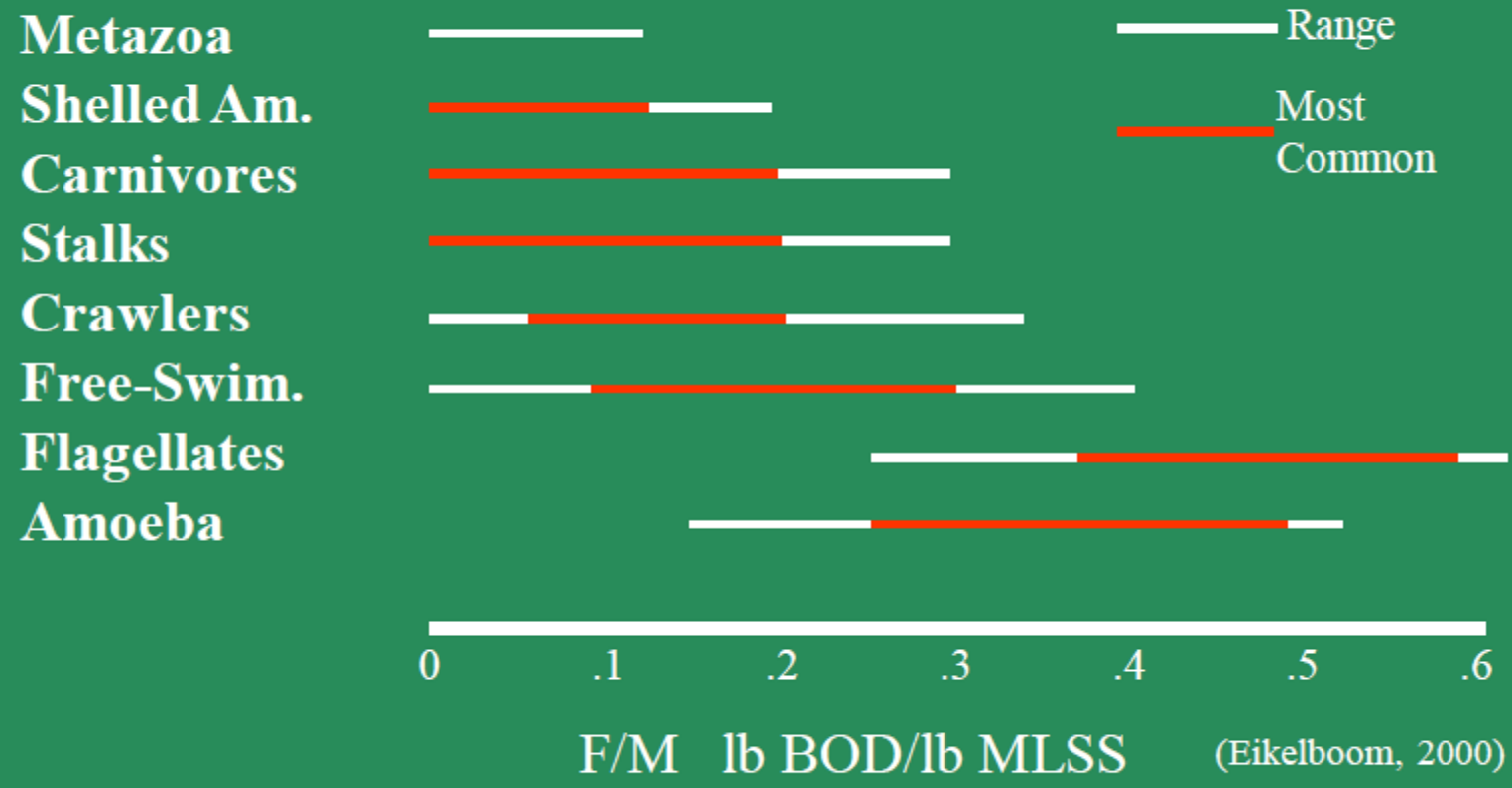


# Stentor



# Water Bear





A light micrograph showing numerous long, thin, filamentous bacteria. The filaments are tangled and some have small, dark, club-like structures at their ends. The background is a light, slightly grainy grey. A central text overlay reads "The Filamentous Bacteria".

# The Filamentous Bacteria

# Filamentous Bacteria

- **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 bacteria. Some filaments are straight, while others are curved or tangled. The bacteria appear as thin, dark lines against a lighter, grainy background.

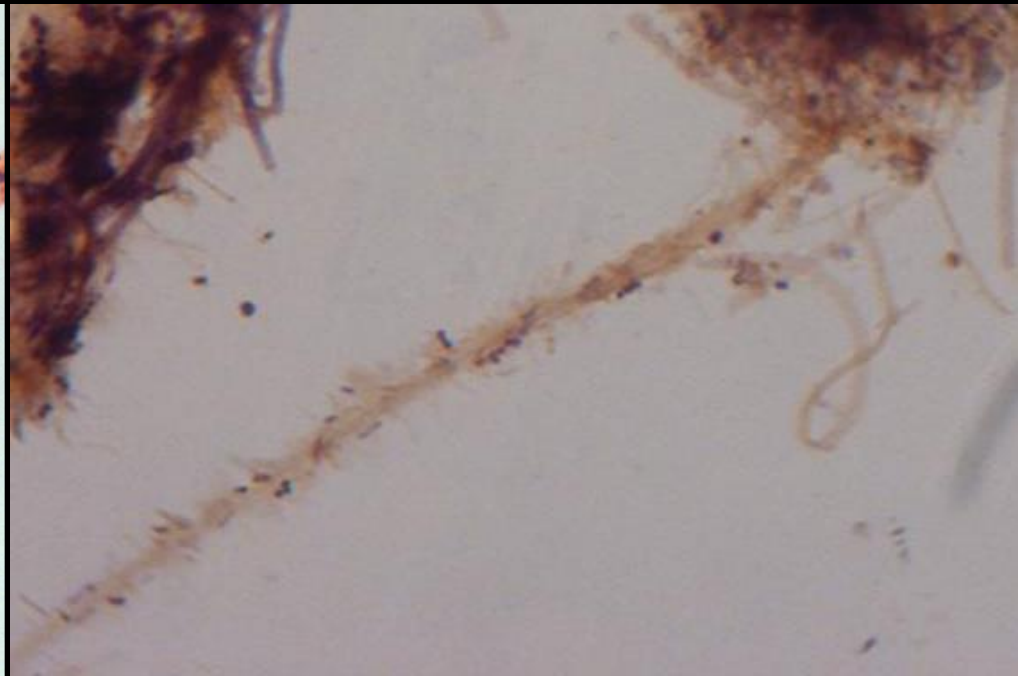
- **Filaments don't lie:**
  - **If you can identify the dominant filaments...**

**...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***

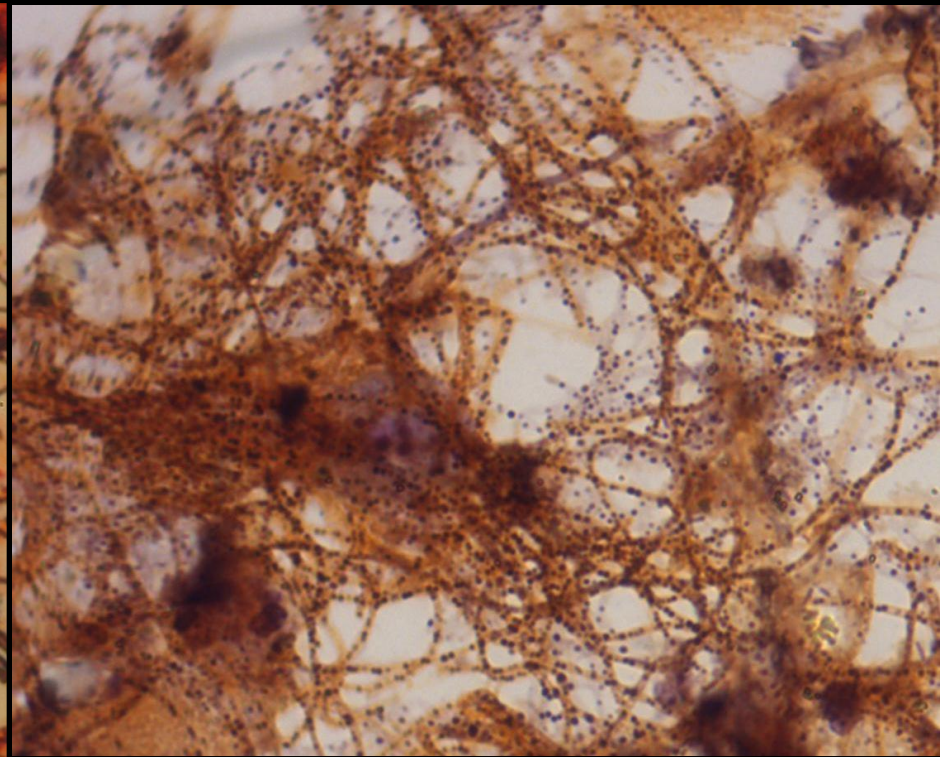
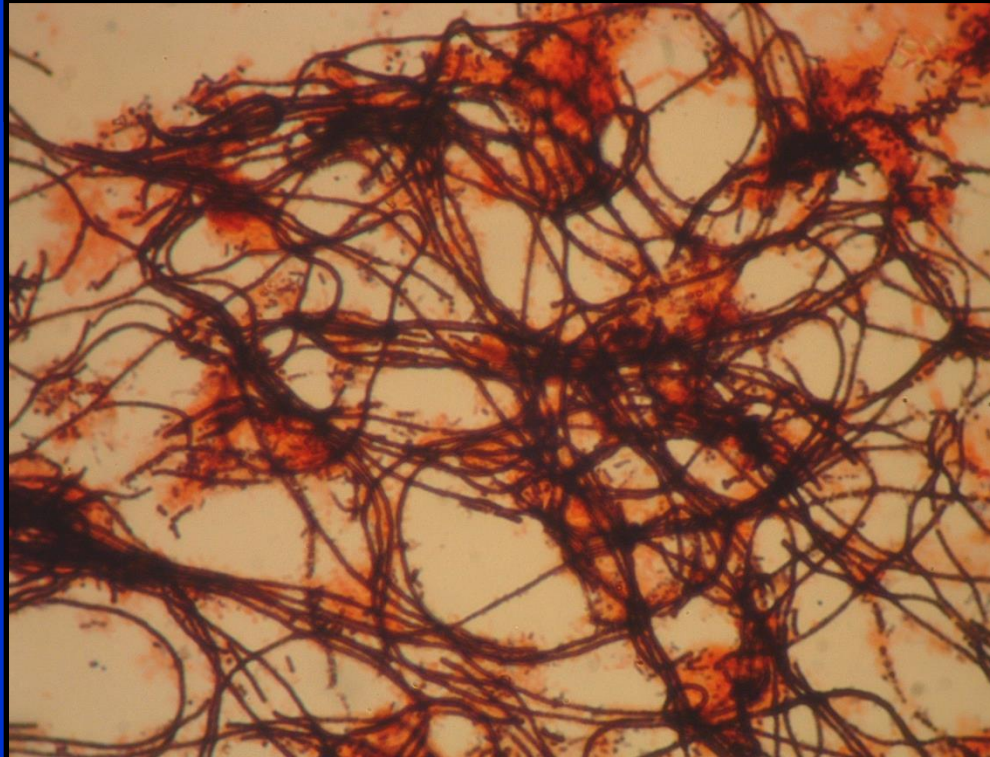


## ***Type 1851***

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

**(Note: Neisser negative difficult to see)**

***Microthrix Parvicella***

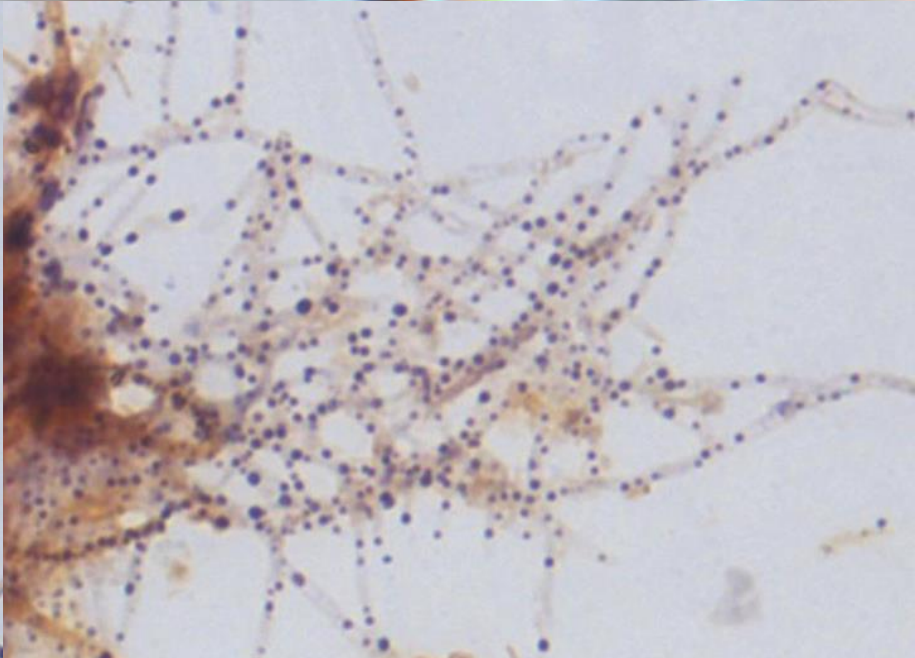
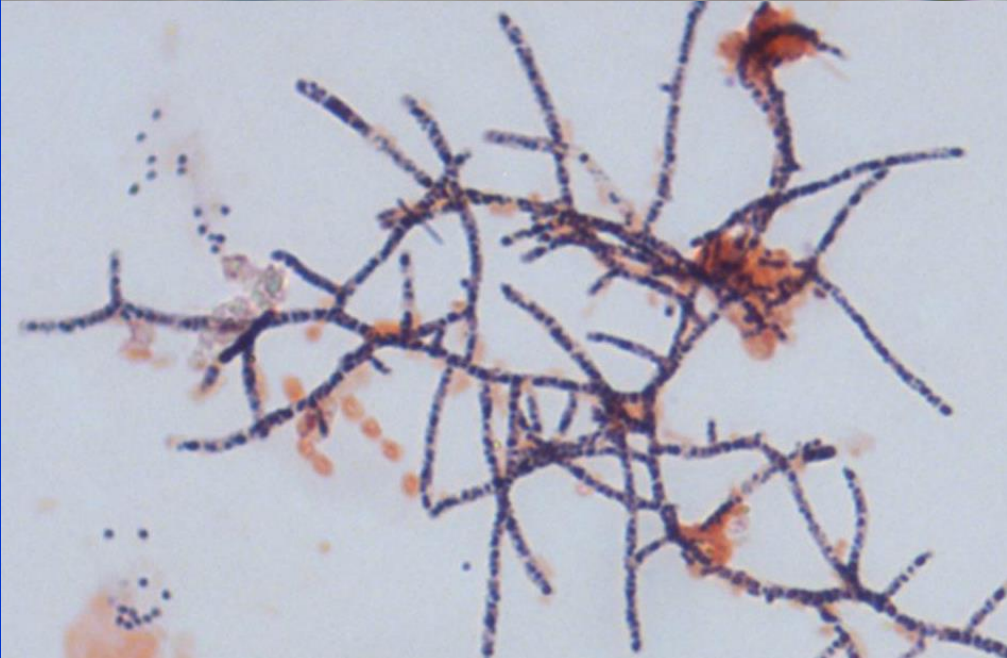
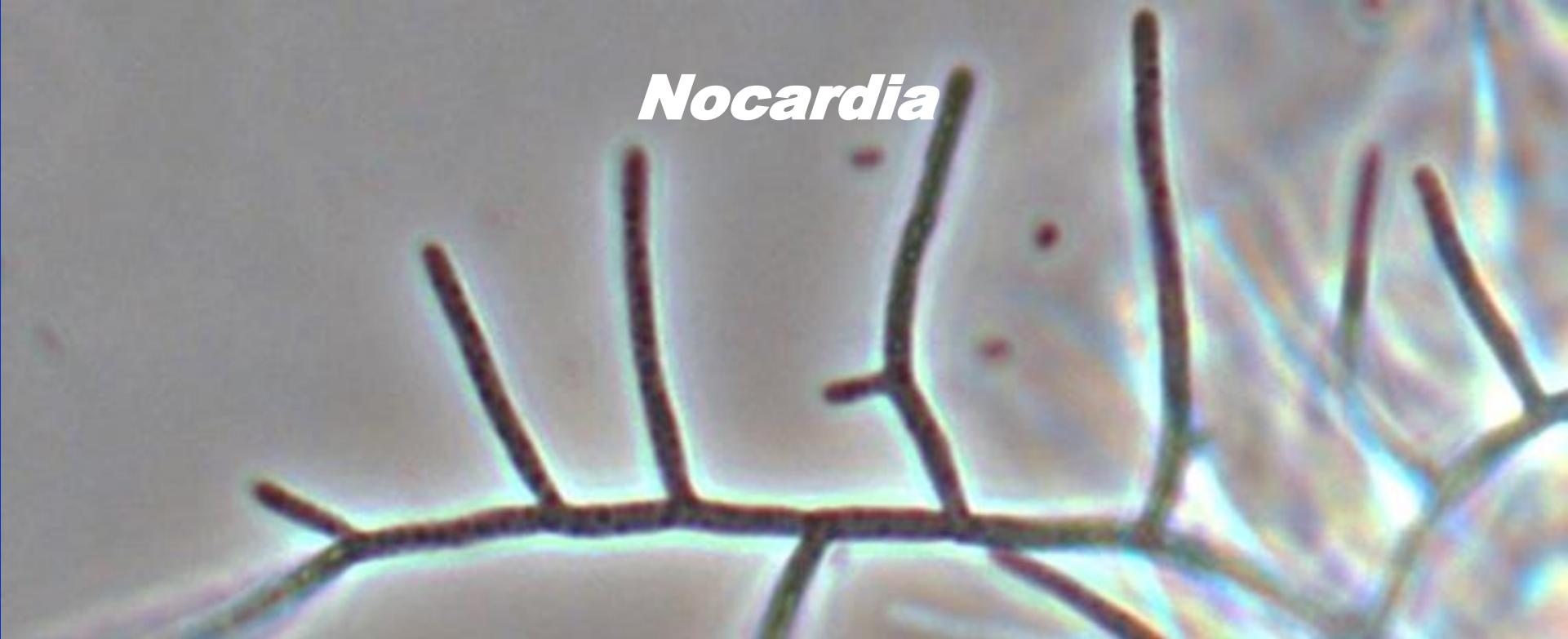


# ***Microthrix Parvicella***

- **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***





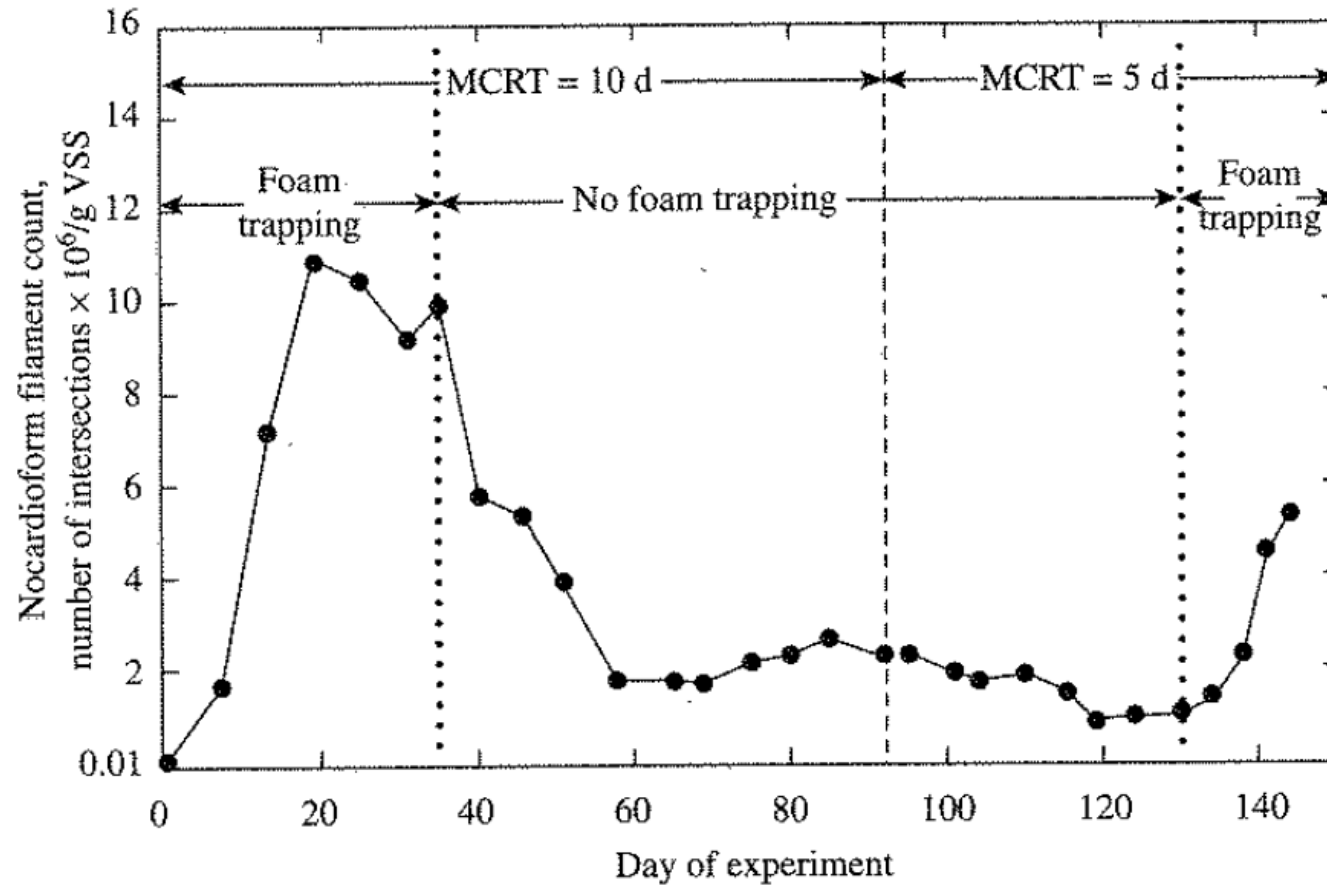
# ***Nocardia***

- **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
  - Increase in-tank pH
  - Waste...a lot

(Note: Neisser positive granules occur)

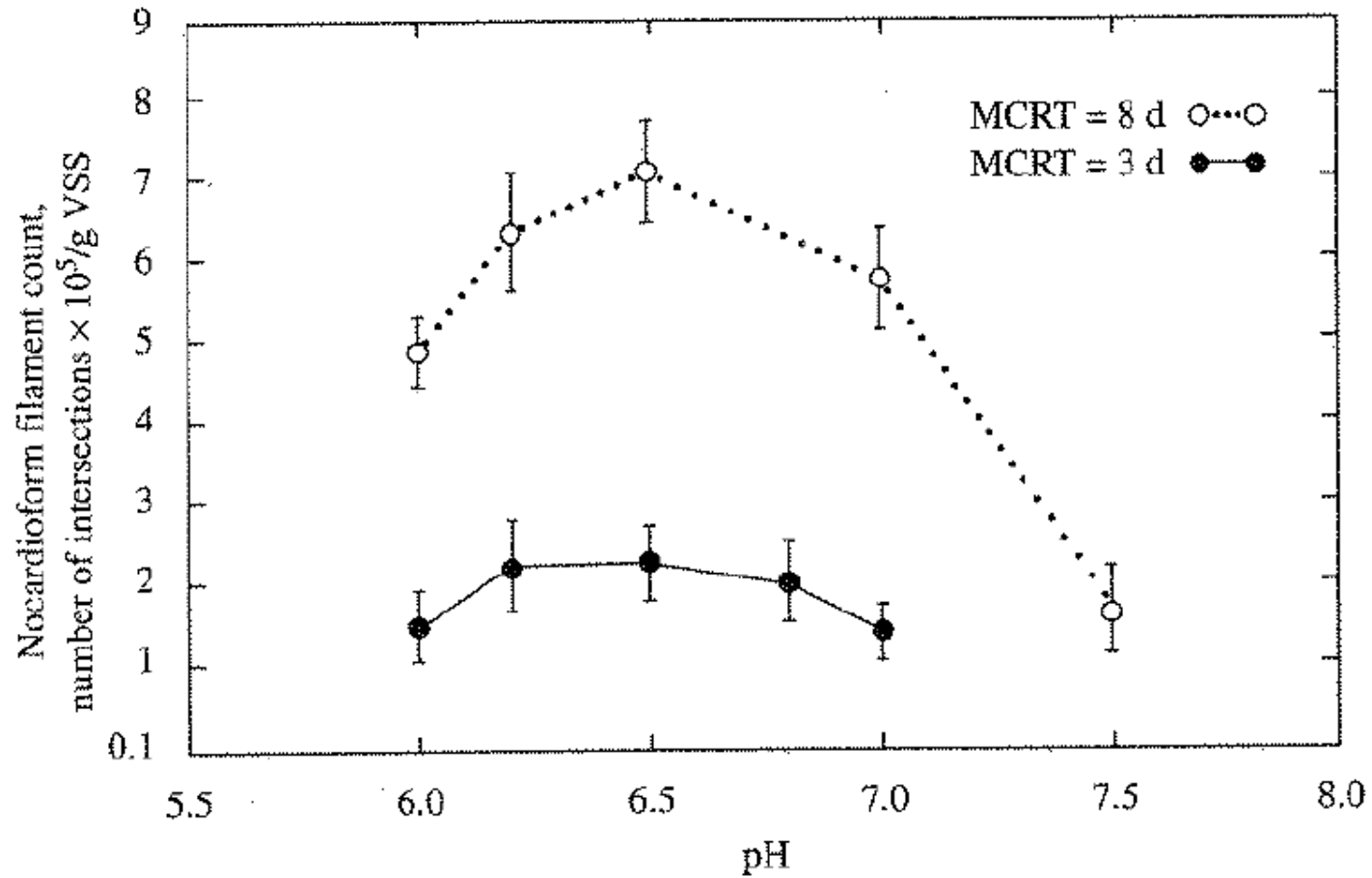


# Foam Trapping



From *Manual on the Causes and Control of Activated Sludge Bulking, Foaming, and other Solids Separation Problem*, Jenkins, D., et al.

# Low pH



From *Manual on the Causes and Control of Activated Sludge Bulking, Foaming, and other Solids Separation Problem*, Jenkins, D., et al.

# Maintain Short MCRT

**TABLE 3.6**  
**Relationship of Specific Filamentous Organisms to MCRT and F/M in Activated Sludge**

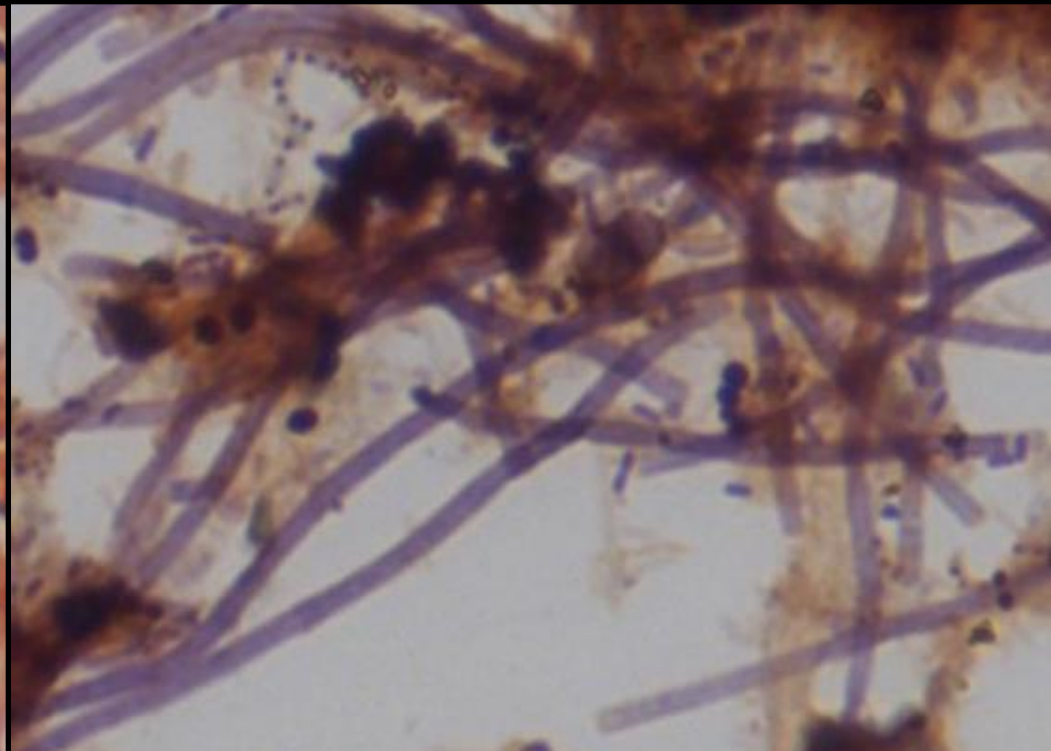
MCRT,d	1.9	2.2	2.5	3.0	4.0	5.0	8.0	20	50
F/M <sup>a</sup>	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	0.05
Type 1701	←-----→								
<i>S. natans</i>	←-----→								
<i>H. hydrossis</i>	←-----→								
<i>Thiothrix</i> spp.	←-----→								
Type 021N	←-----→								
Nocardioforms	←-----→								
Type 0411	←-----→								
<i>N. limicola</i> II	←-----→								
Type 1863	←-----→								
Type 0041	←-----→								
Type 0675	←-----→								
<i>M. parvicella</i>	←-----→								
Type 0092	←-----→								
Type 1851	←-----→								
Type 0914	←-----→								
Type 0803	←-----→								
Type 0581	←-----→								

<sup>a</sup> F/M as kg BOD<sub>5</sub>/kg MLSS, d.

Sources: From Richard, M.G. (1989), *Activated Sludge Microbiology*, Water Pollution Control Federation, Alexandria VA and Eikelboom, D.H. (2000), *Process Control of Activated Sludge Plants by Microscopic Investigation*, IWA Publishing, London.



***Type 0092***



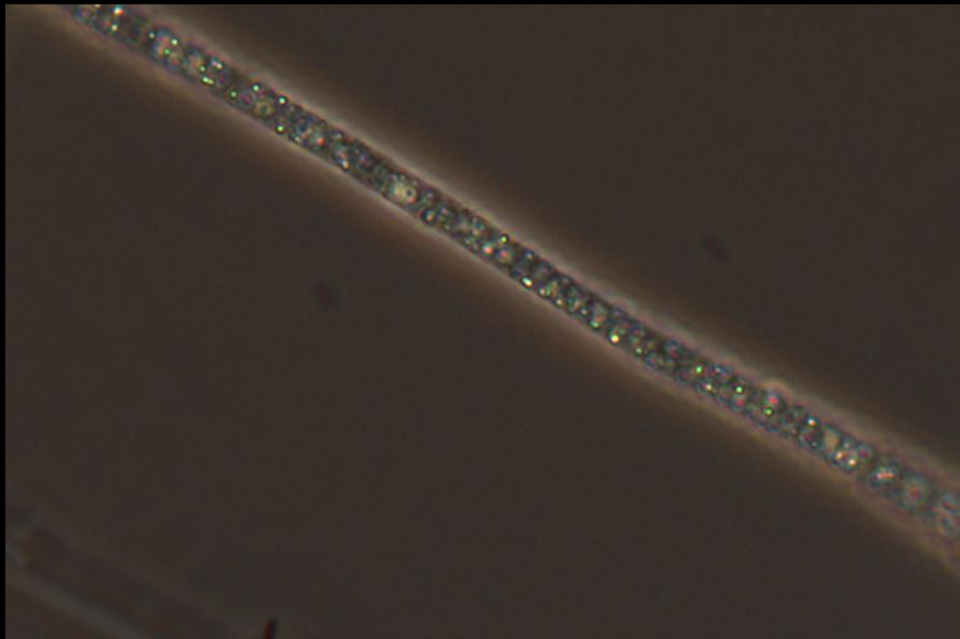
## ***Type 0092***

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

(Note: Gram negative difficult to see)



# Thiothrix

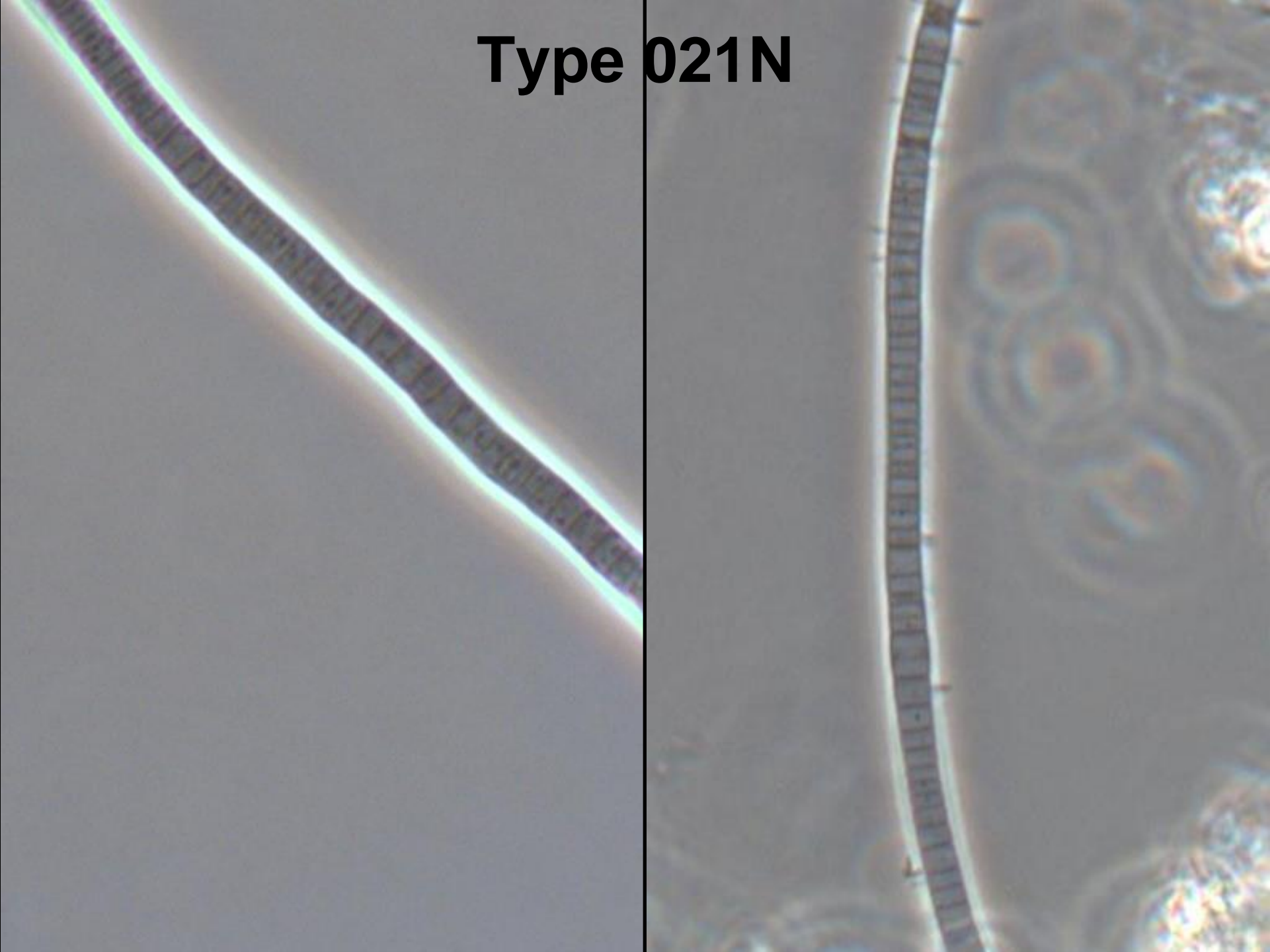


# ***Thiothrix***

A microscopic image showing numerous long, thin, filamentous structures of Thiothrix bacteria. The filaments are tangled and some have small, rounded, bulbous structures attached to them. The background is a light, slightly grainy grey.

- **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

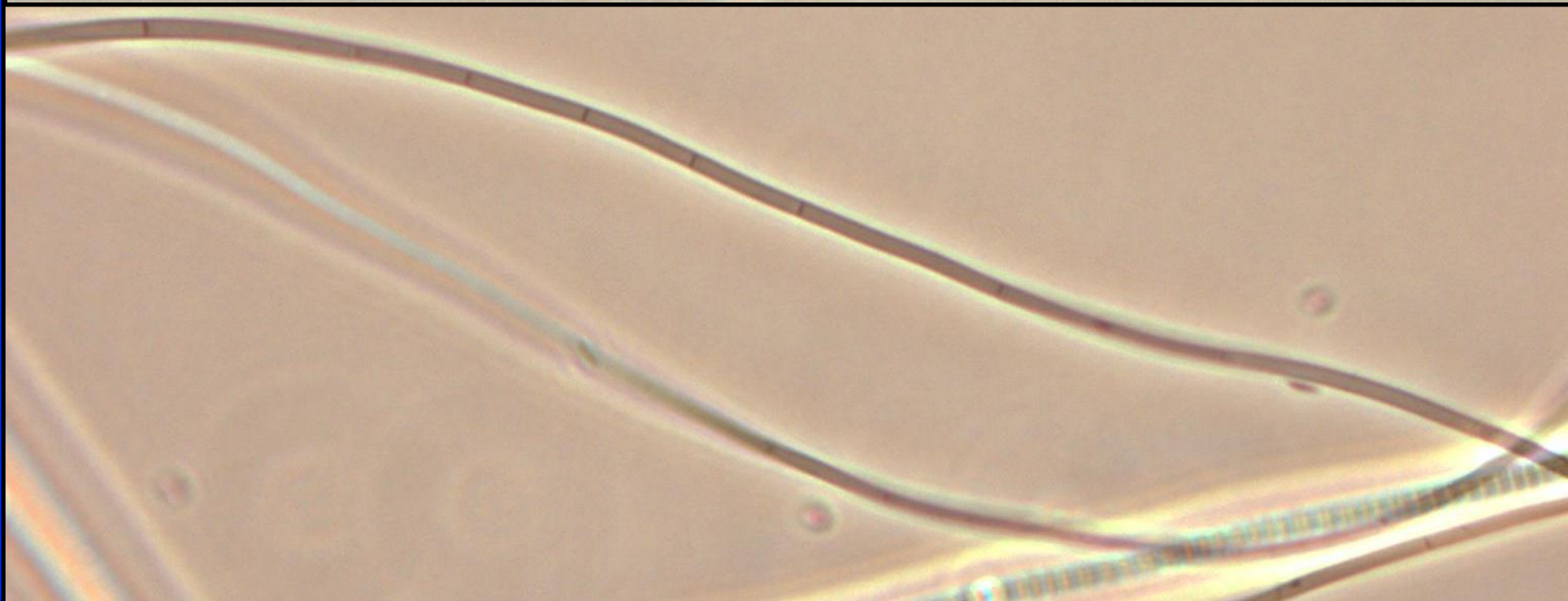
Type 021N



## ***Type 021N***

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

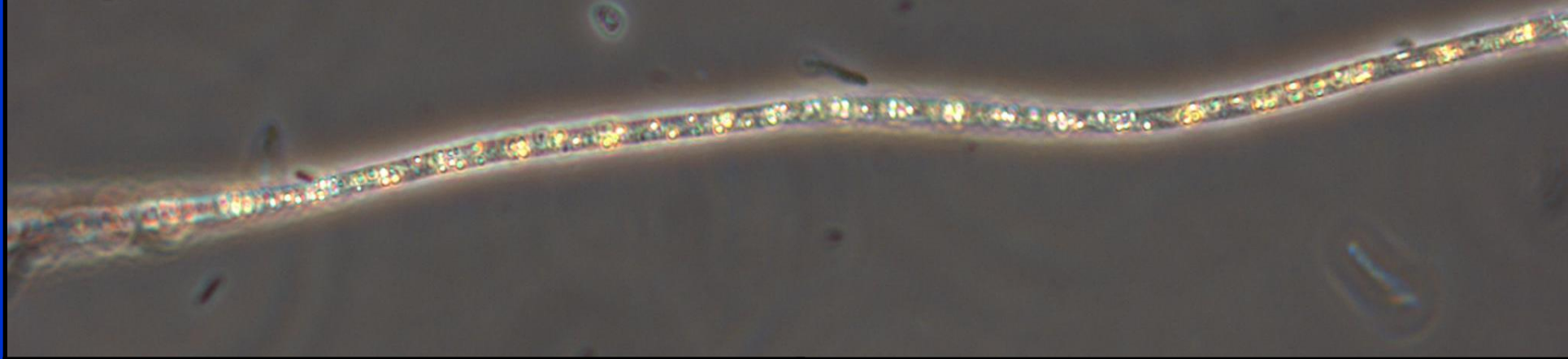
**Type 0961**



## ***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***

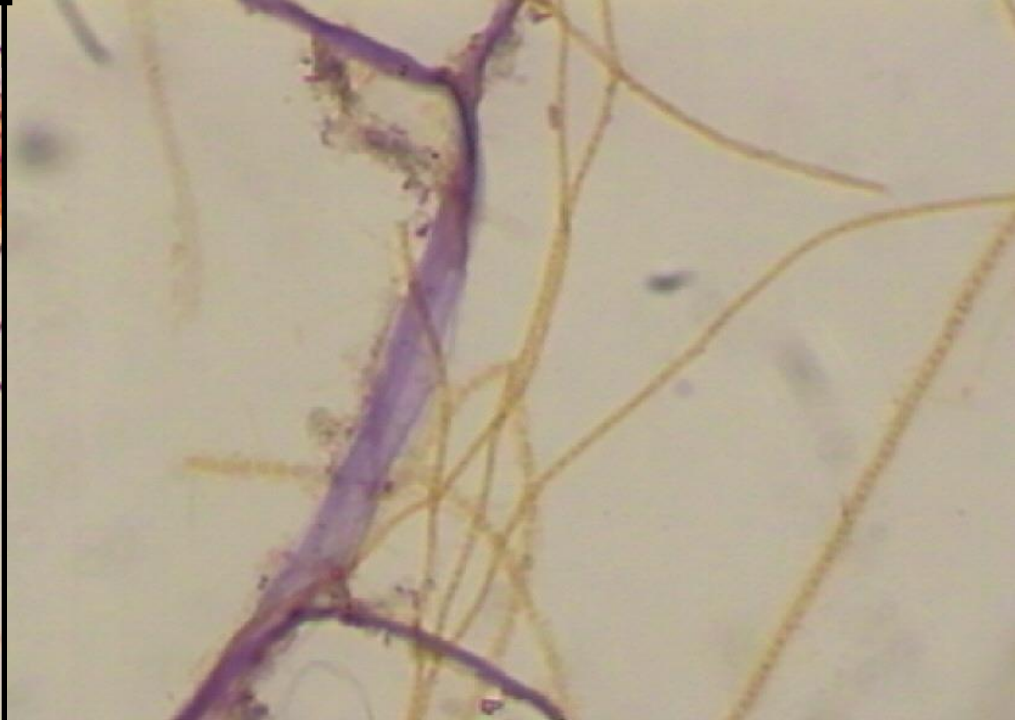
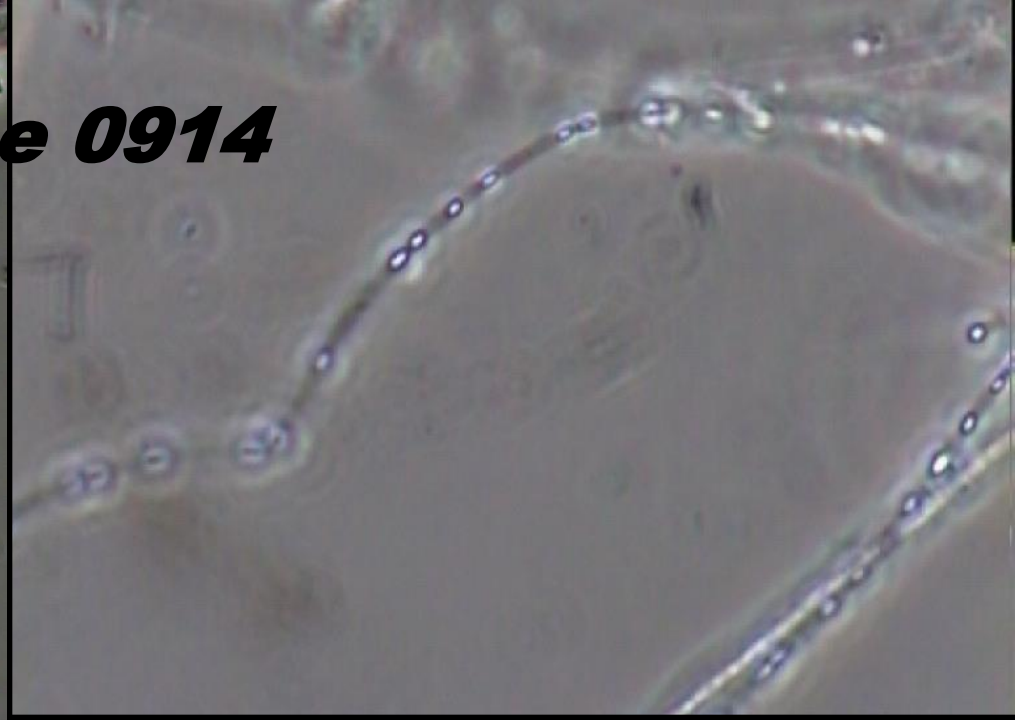


# ***Nostocoida Limicola***

- **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

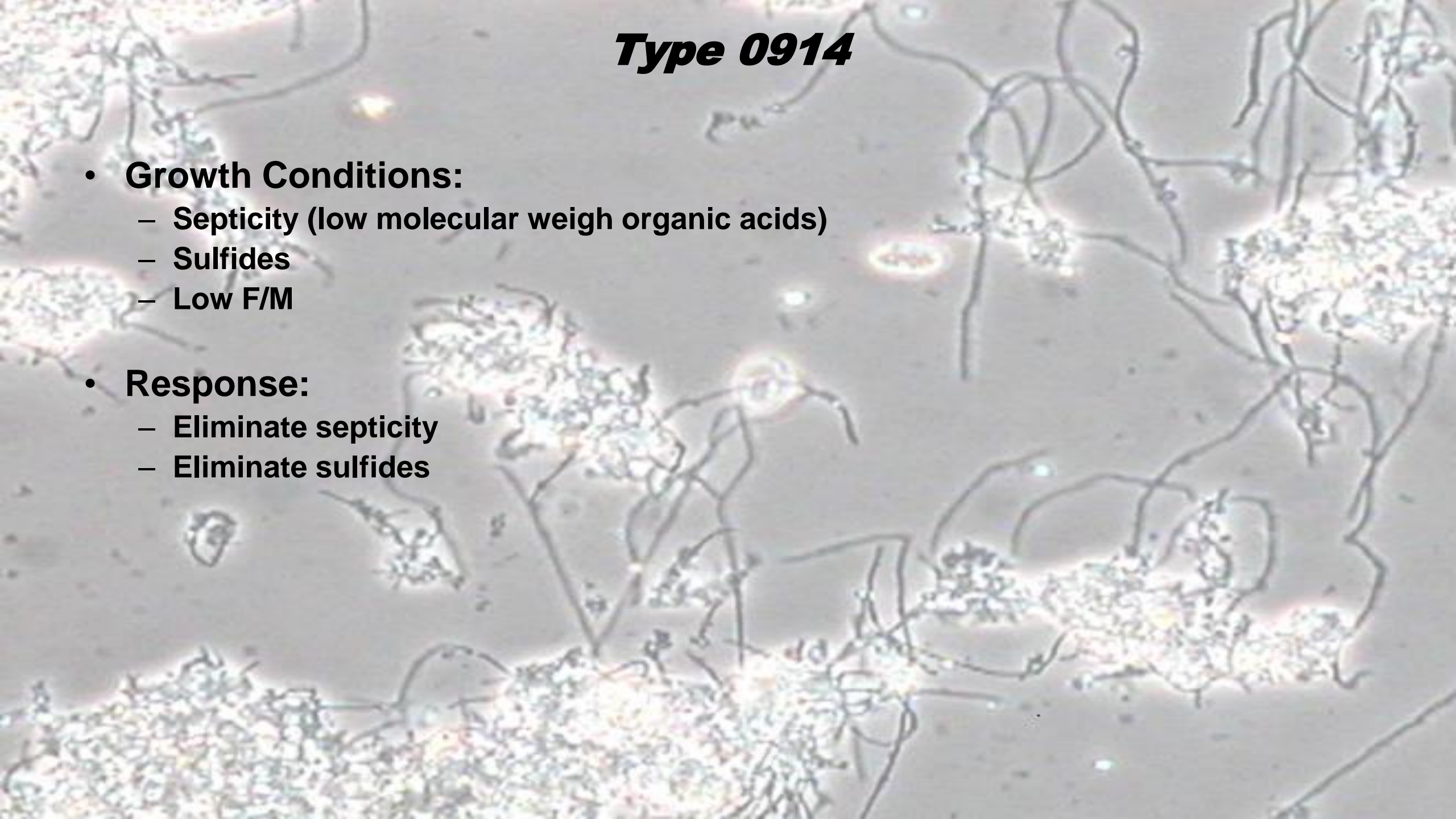
(Note: Gram and Neisser negative occur)

***Type 0914***

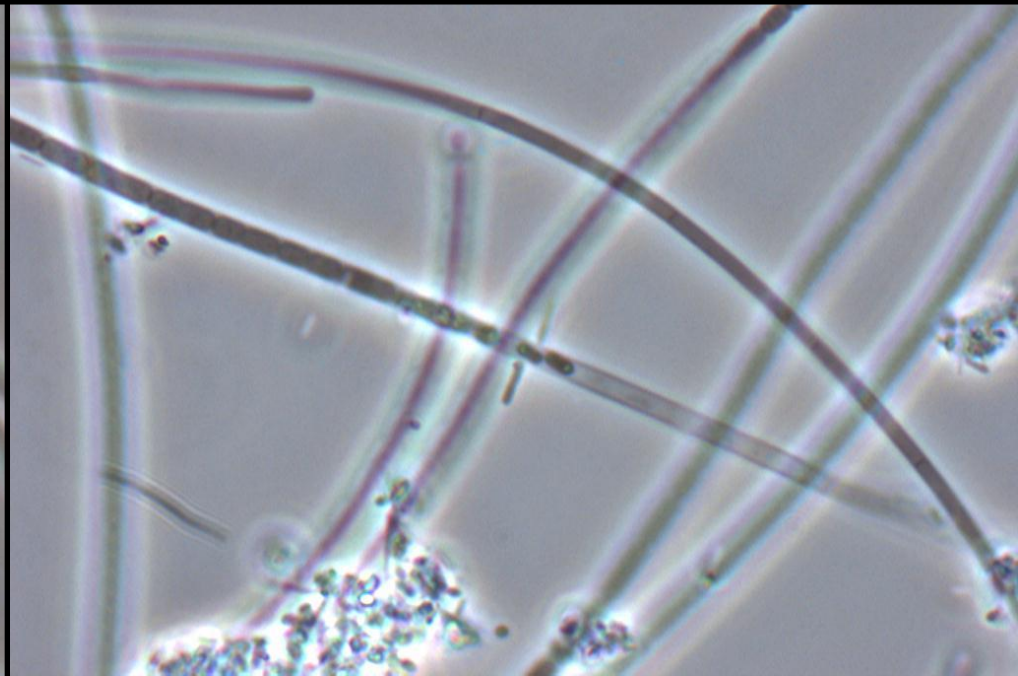
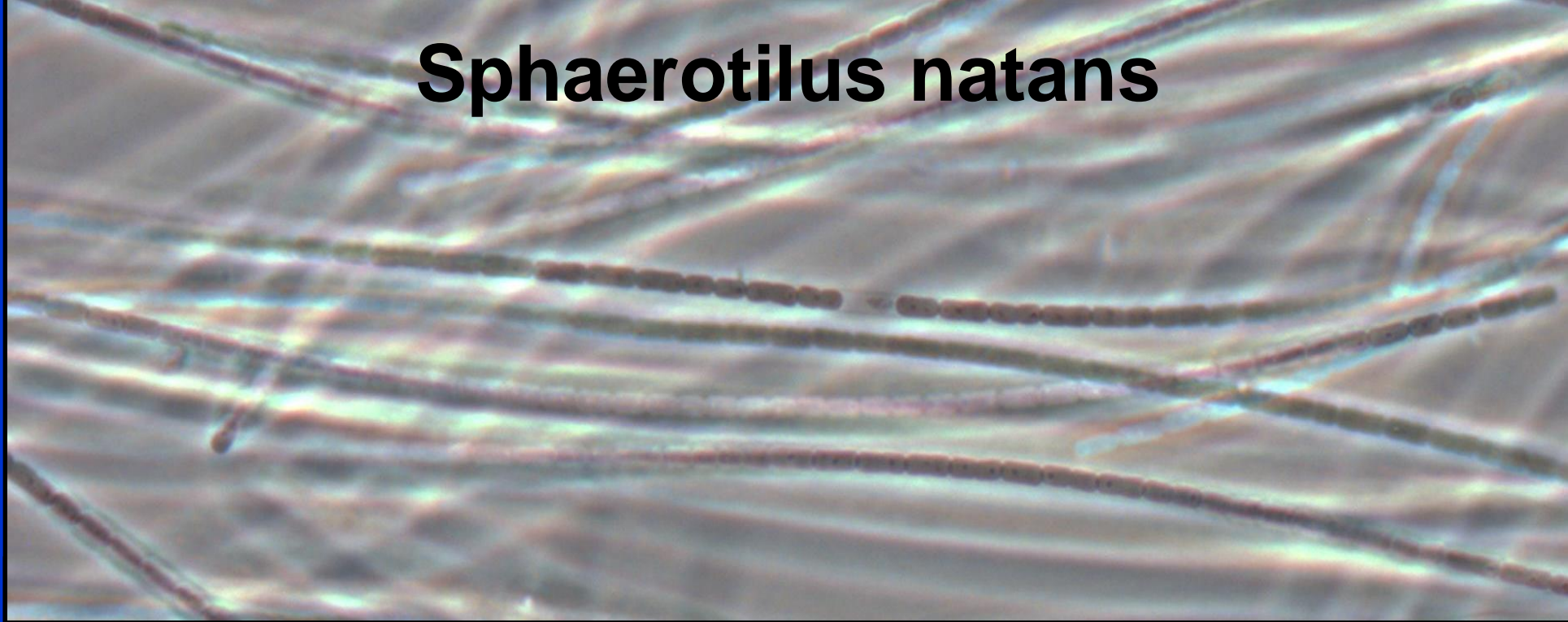


## ***Type 0914***

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



# Sphaerotilus natans

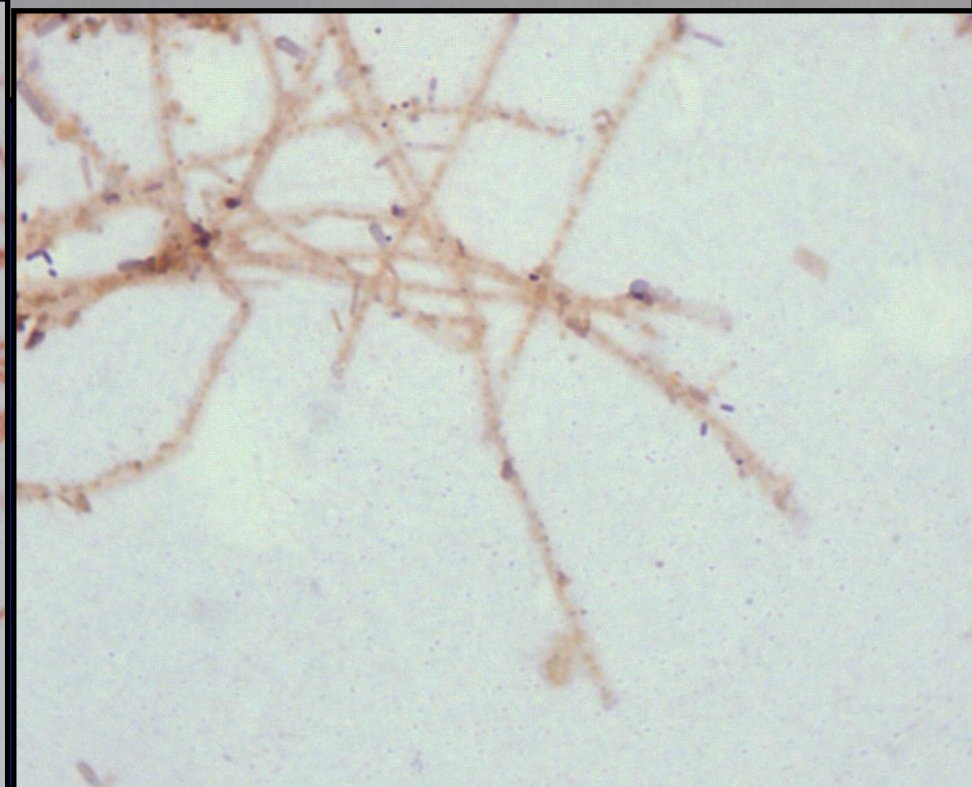
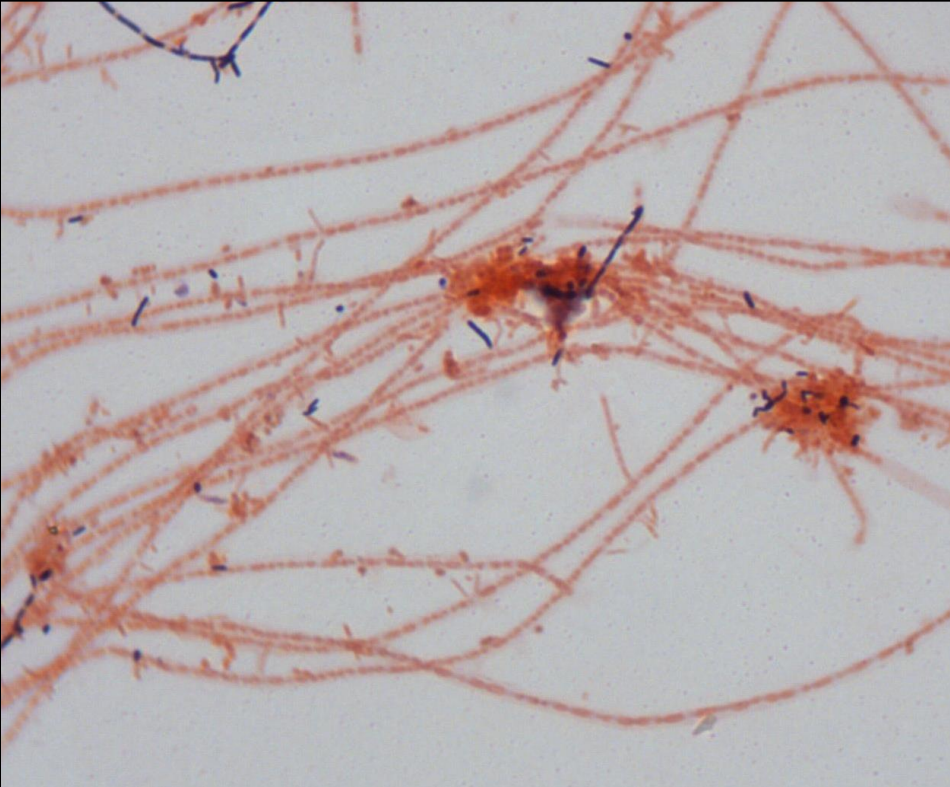
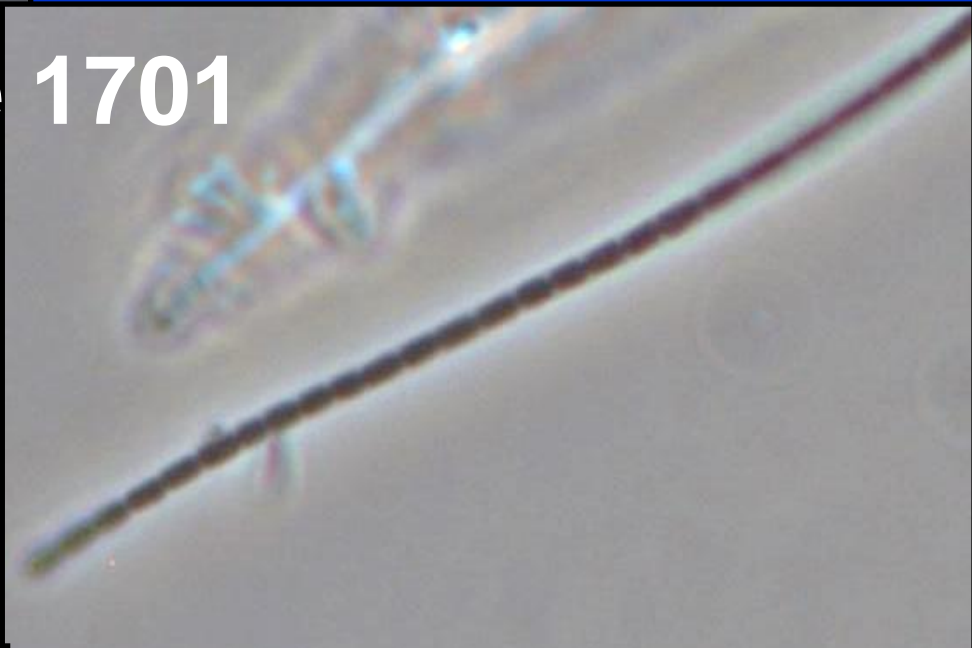
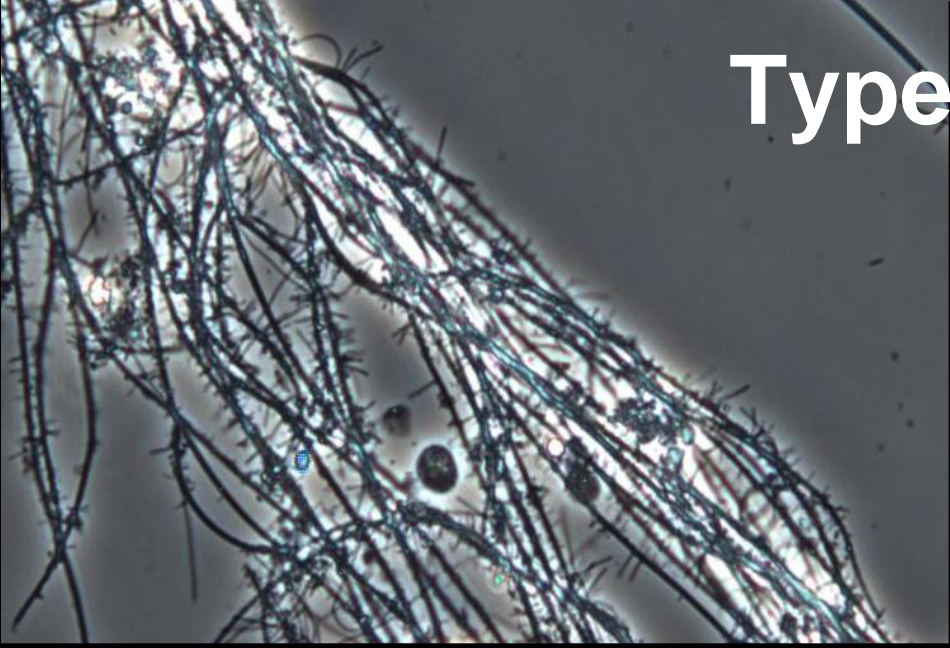


# ***Sphaerotilus natans***

A microscopic image showing numerous long, thin, filamentous structures of Sphaerotilus natans. The filaments are tangled and appear to be composed of individual cells. 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

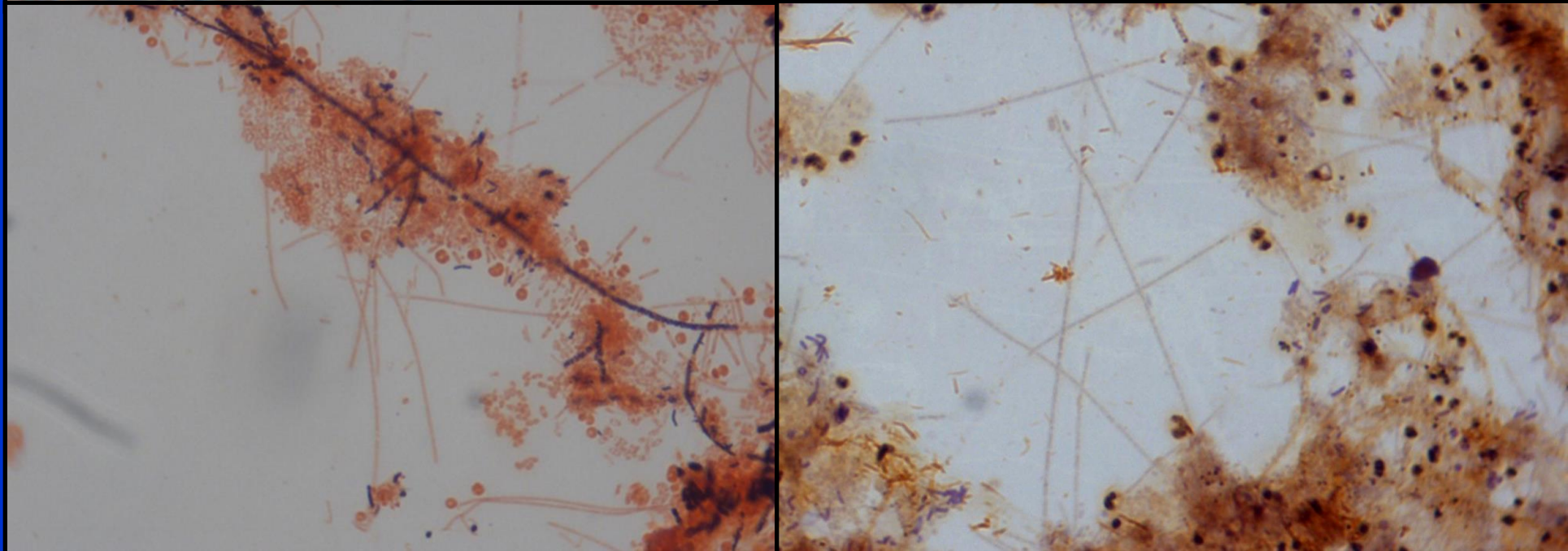
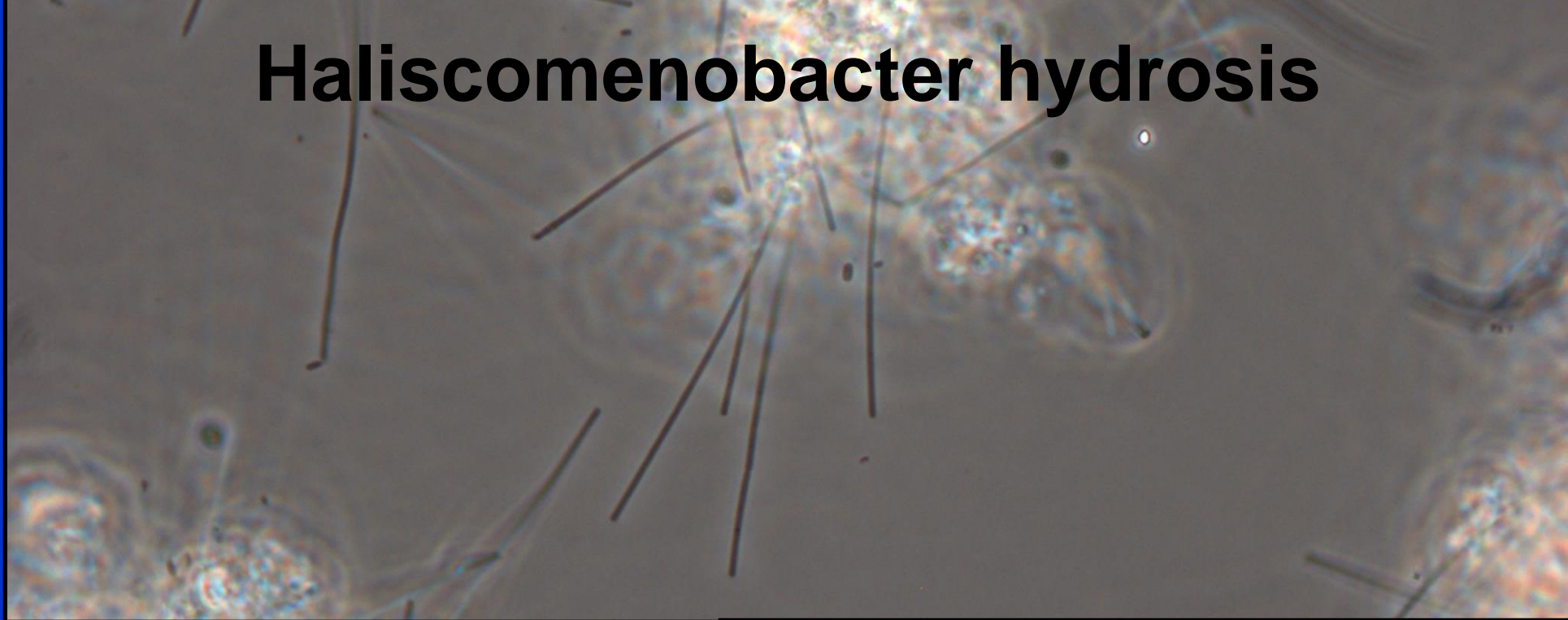


## ***Type 1701***

- **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

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



**Staining Techniques:  
Reveal What is Hidden**

A microscopic image showing a dense field of bacteria. The bacteria appear as small, dark, rod-shaped structures, some of which are arranged in chains or clusters. The background is a light, slightly grainy grey. Overlaid on the center of the image is the text "Why Stain the Bacteria?" in a bold, black, sans-serif font.

**Why Stain the Bacteria?**









# 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**
- **Sink (preferably a black lab sink) or roasting pan**
- **Bon Ami Scouring Powder (optional)**

# Tools for Staining



- **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

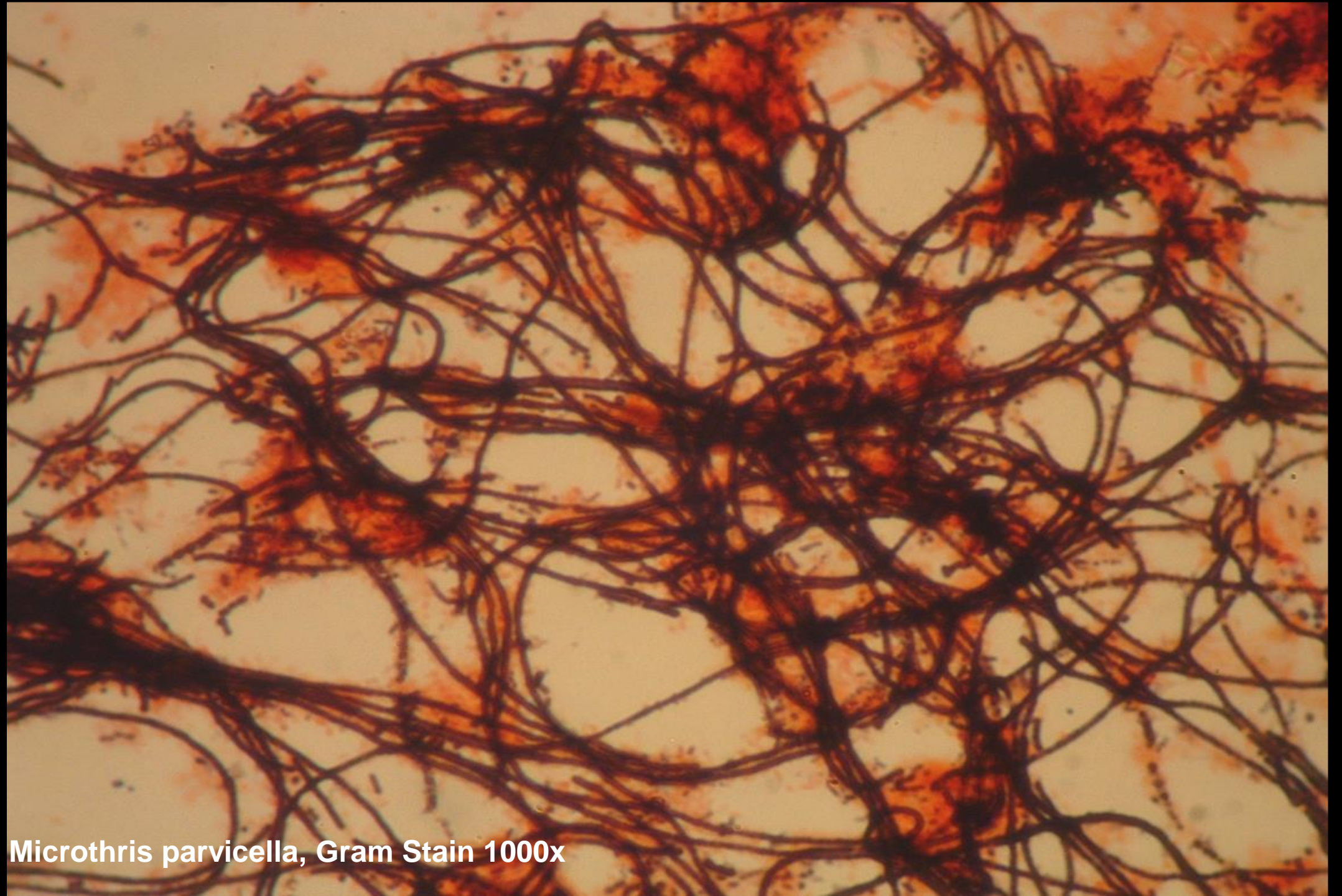
## 3. Gram Decolorizing Solution

- Hold slide at 45 degrees and apply dropwise until blue color stops rinsing off (15-20 seconds max)
- Blast with DI water to stop reaction, blot dry with paper towel

## 4. Gram Safranin Solution

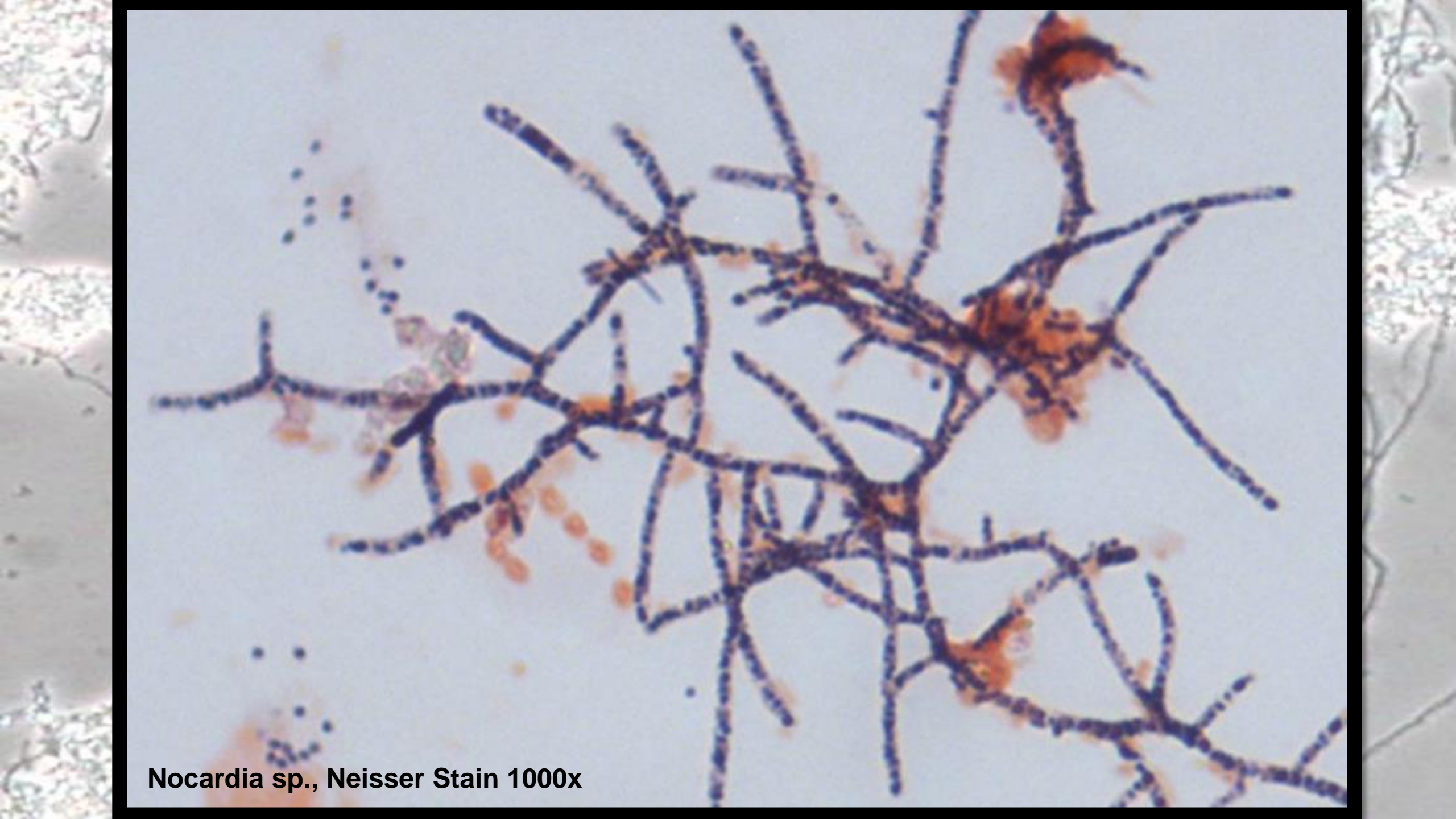
- Flood slide for 1 minute
- Rinse with DI water

## 5. View Slide at 1000x under bright light (not phase contrast)



**Microthrix parvicella, Gram Stain 1000x**





**Nocardia sp., Neisser Stain 1000x**

# 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**
  - **And the Clothes Pin, of course**

# Neisser Staining Procedure

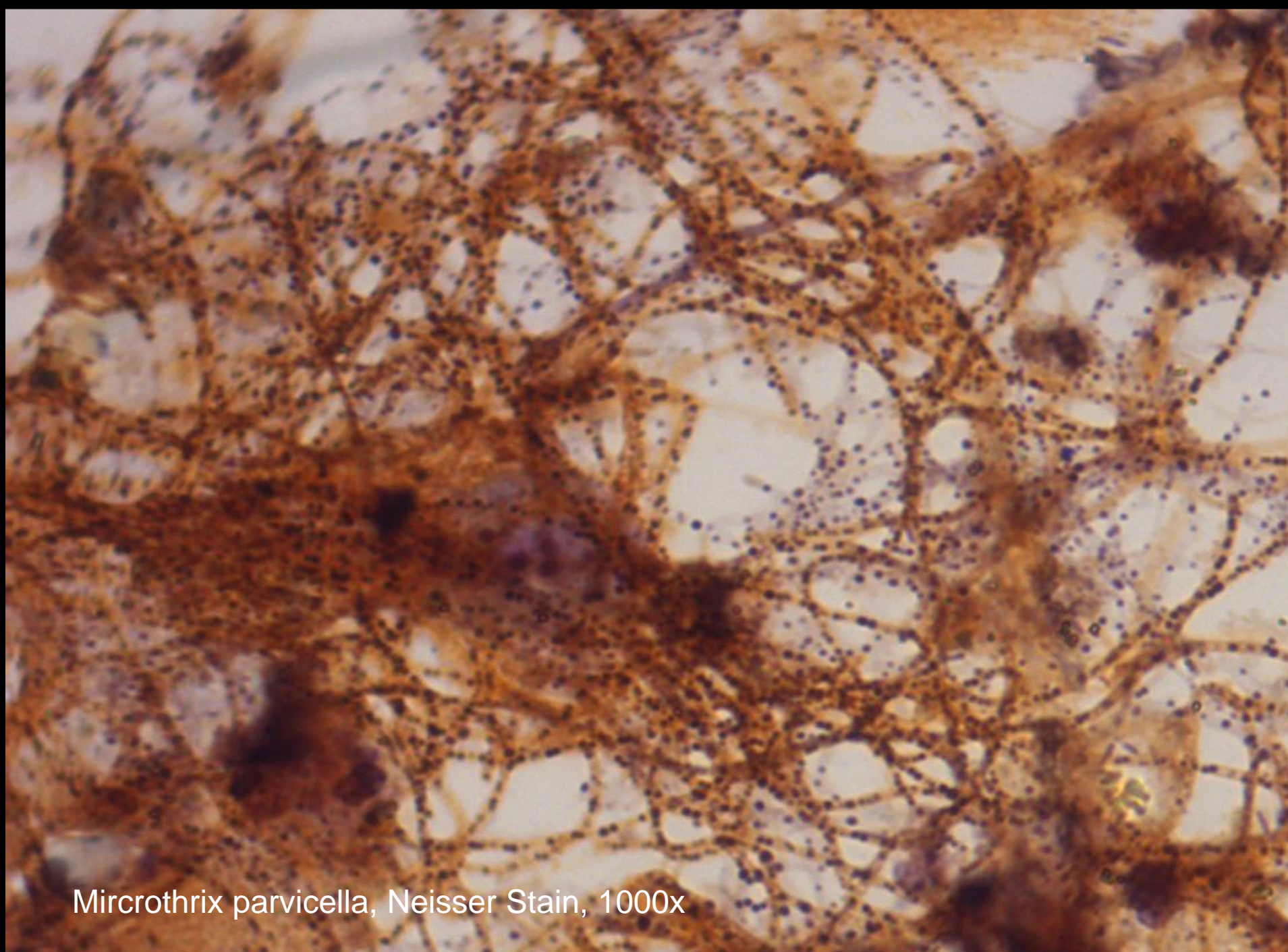
## 1. Methyl Blue / Crystal Violet Solution

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

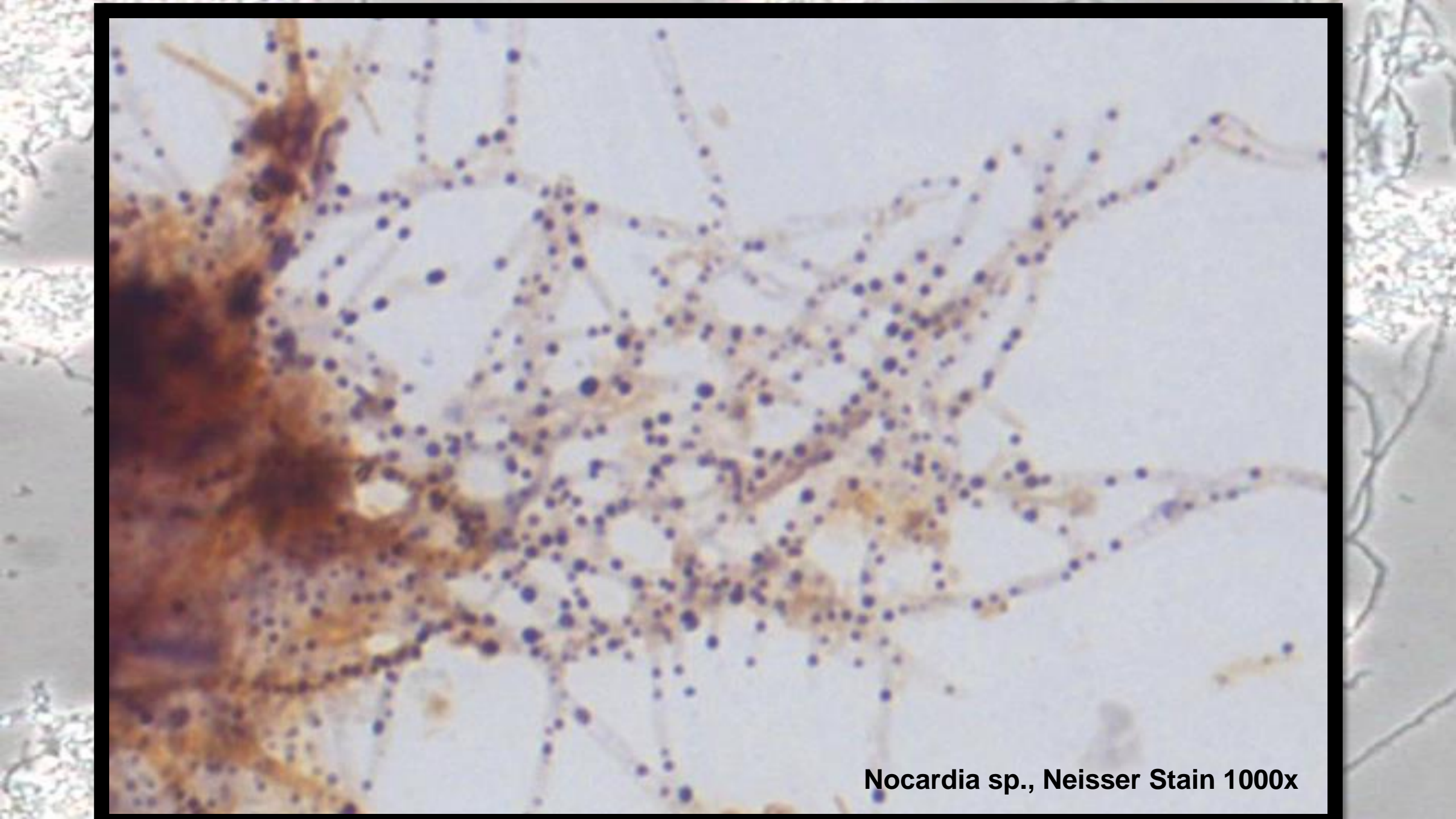
## 2. Bismark Brown Solution

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

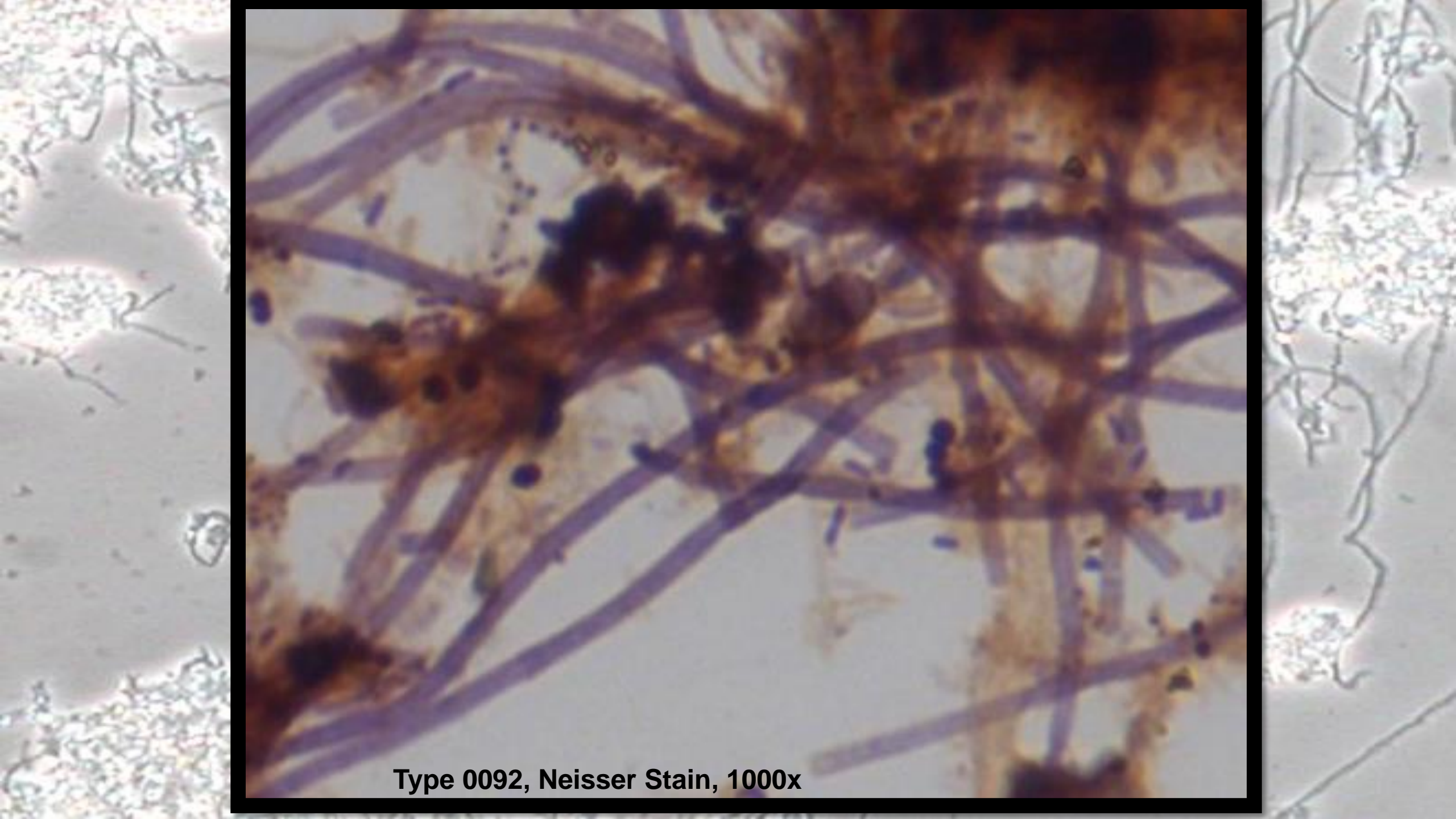
## 3. View Slide at 1000x bright light (not phase contrast)



*Microthrix parvicella*, Neisser Stain, 1000x



**Nocardia sp., Neisser Stain 1000x**



Type 0092, Neisser Stain, 1000x

A microscopic view of a bacterial culture, showing various cell shapes and structures. The background is a light, grainy texture with several distinct clusters and individual cells. Some cells appear as small, dark, rounded shapes, while others are more elongated and filamentous. The overall appearance is that of a complex microbial community.

# **Why Stain the Bacteria?**

- 1) Staining is actually very easy**
- 2) Staining bacteria will help determine what is growing**
- 3) Staining will show what is hidden in a wet mount**
- 4) Staining can be effective if a phase contrast microscope is not available**

A microscopic image of plant tissue, showing a network of thin, light-colored cell walls and larger, more complex structures. The background is a light, slightly grainy grey. Overlaid on this image is bold black text.

**Questions?**

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