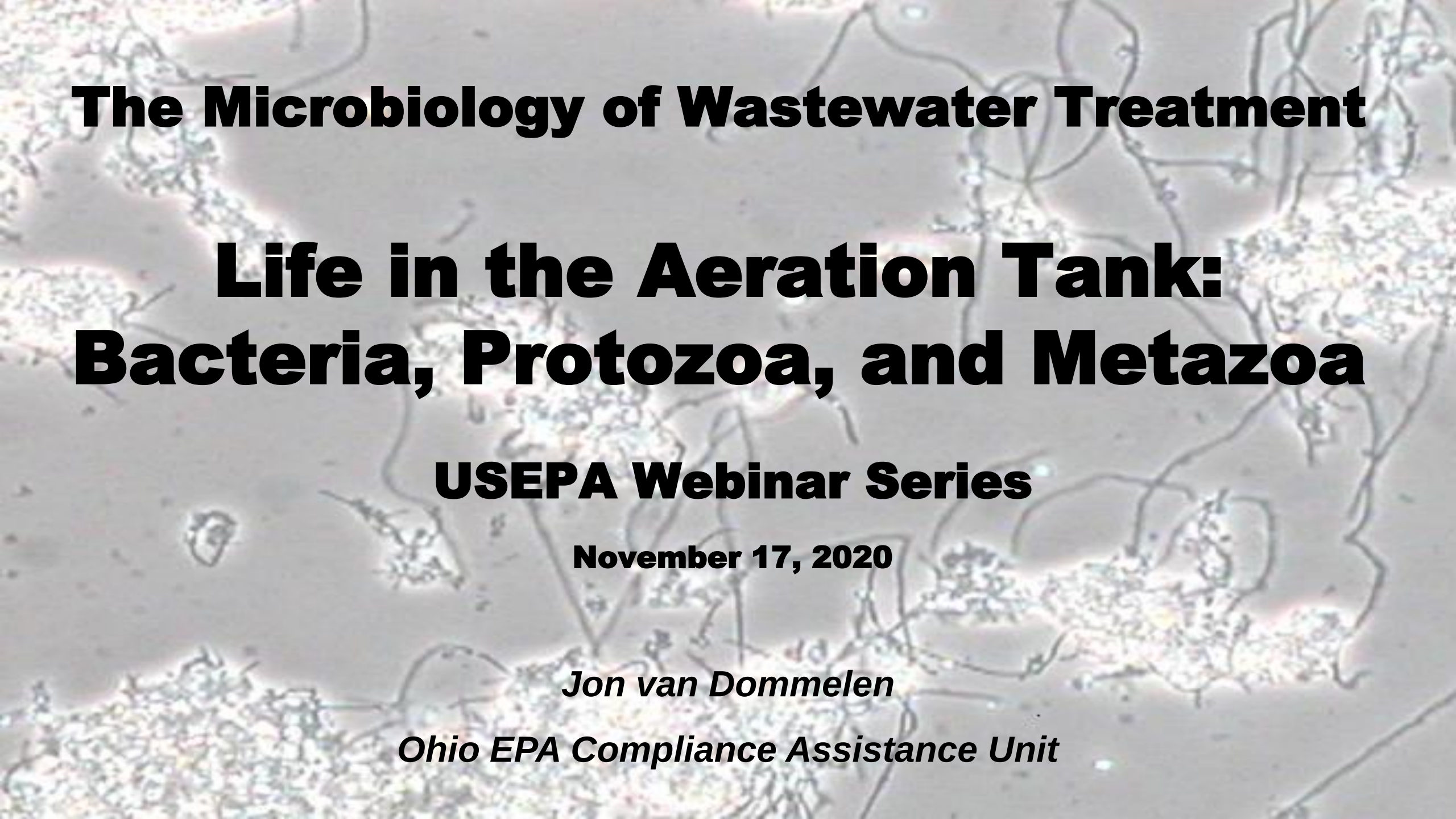


The Microbiology of Wastewater Treatment

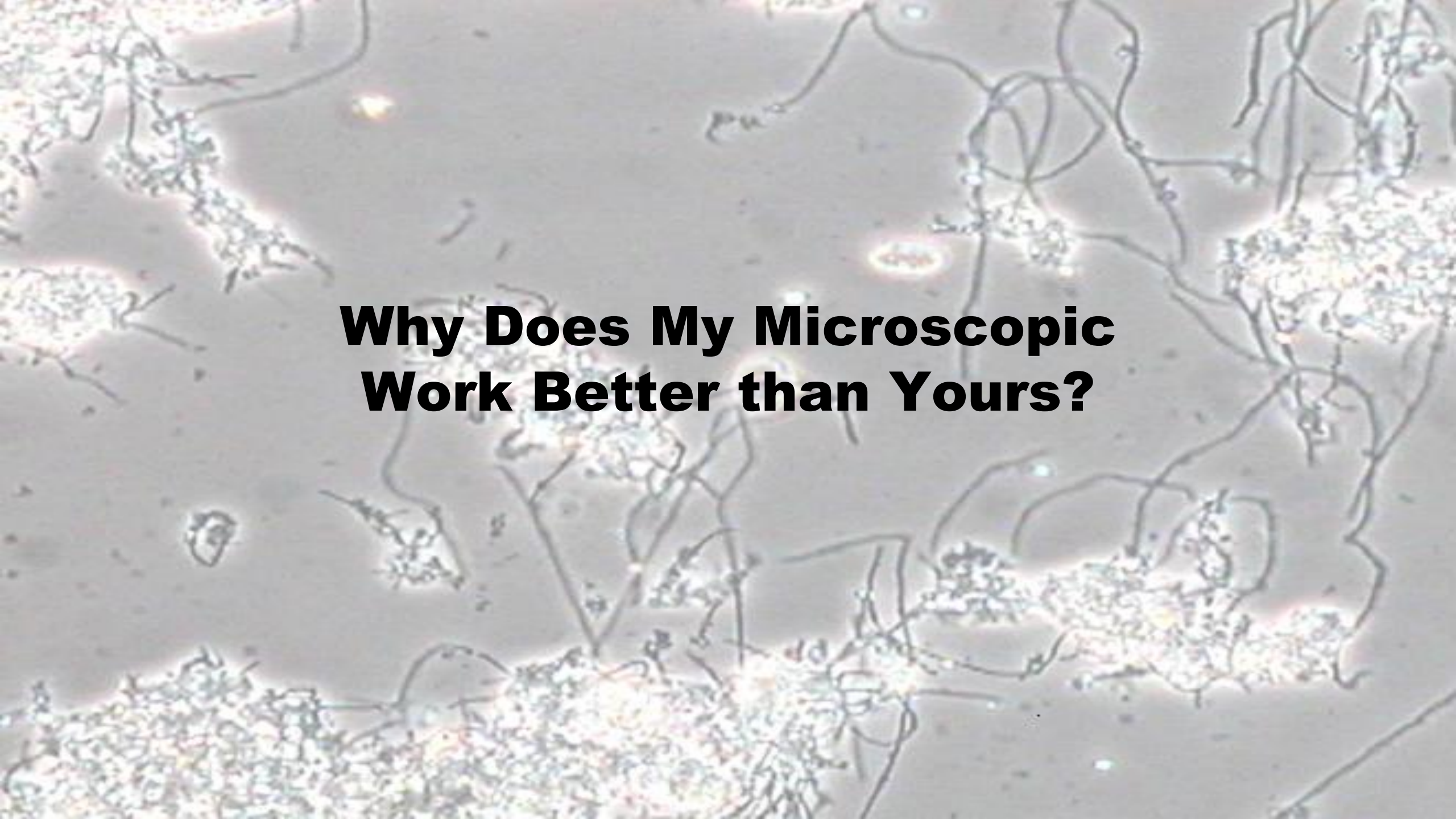
Life in the Aeration Tank: Bacteria, Protozoa, and Metazoa

USEPA Webinar Series

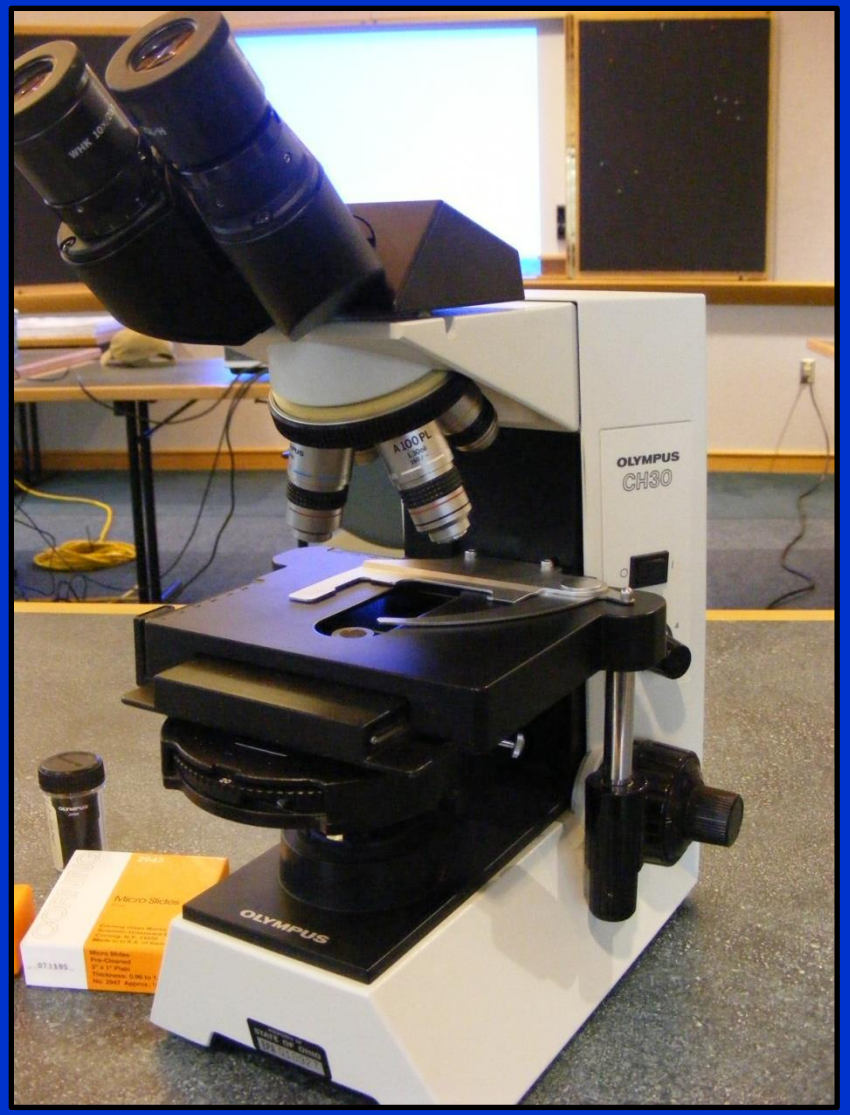
November 17, 2020

Jon van Dommelen

Ohio EPA Compliance Assistance Unit



Why Does My Microscopic Work Better than Yours?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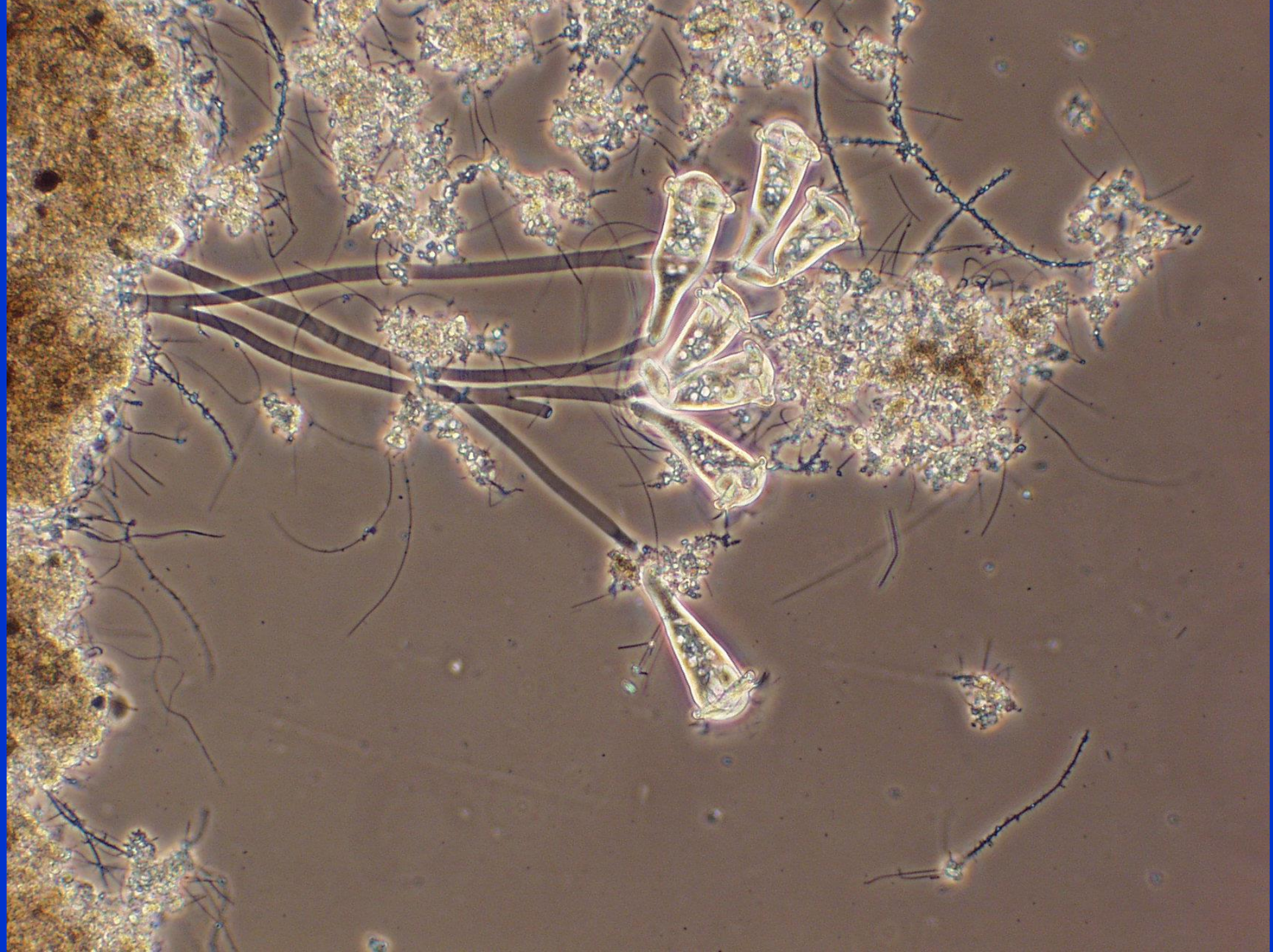
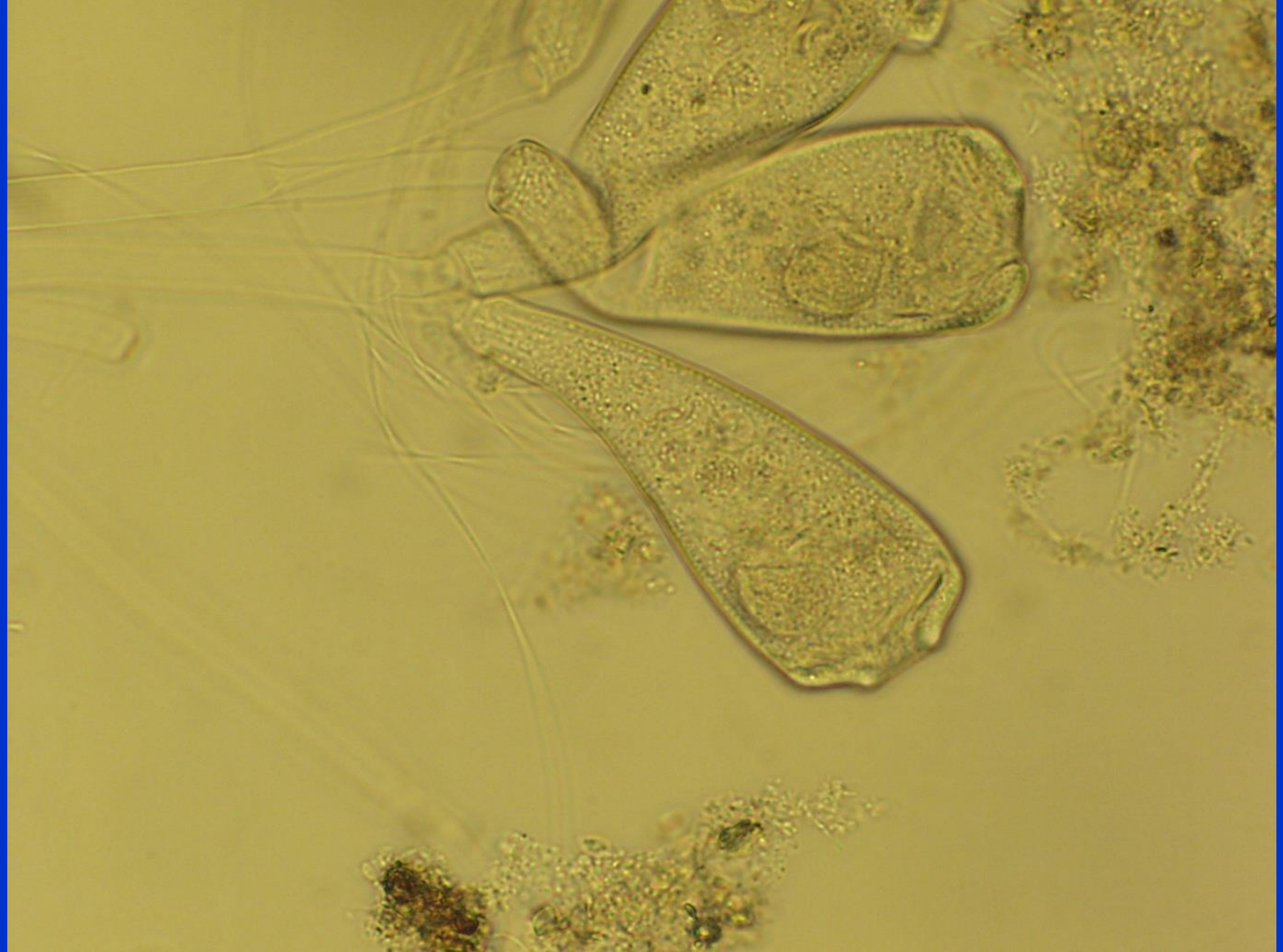
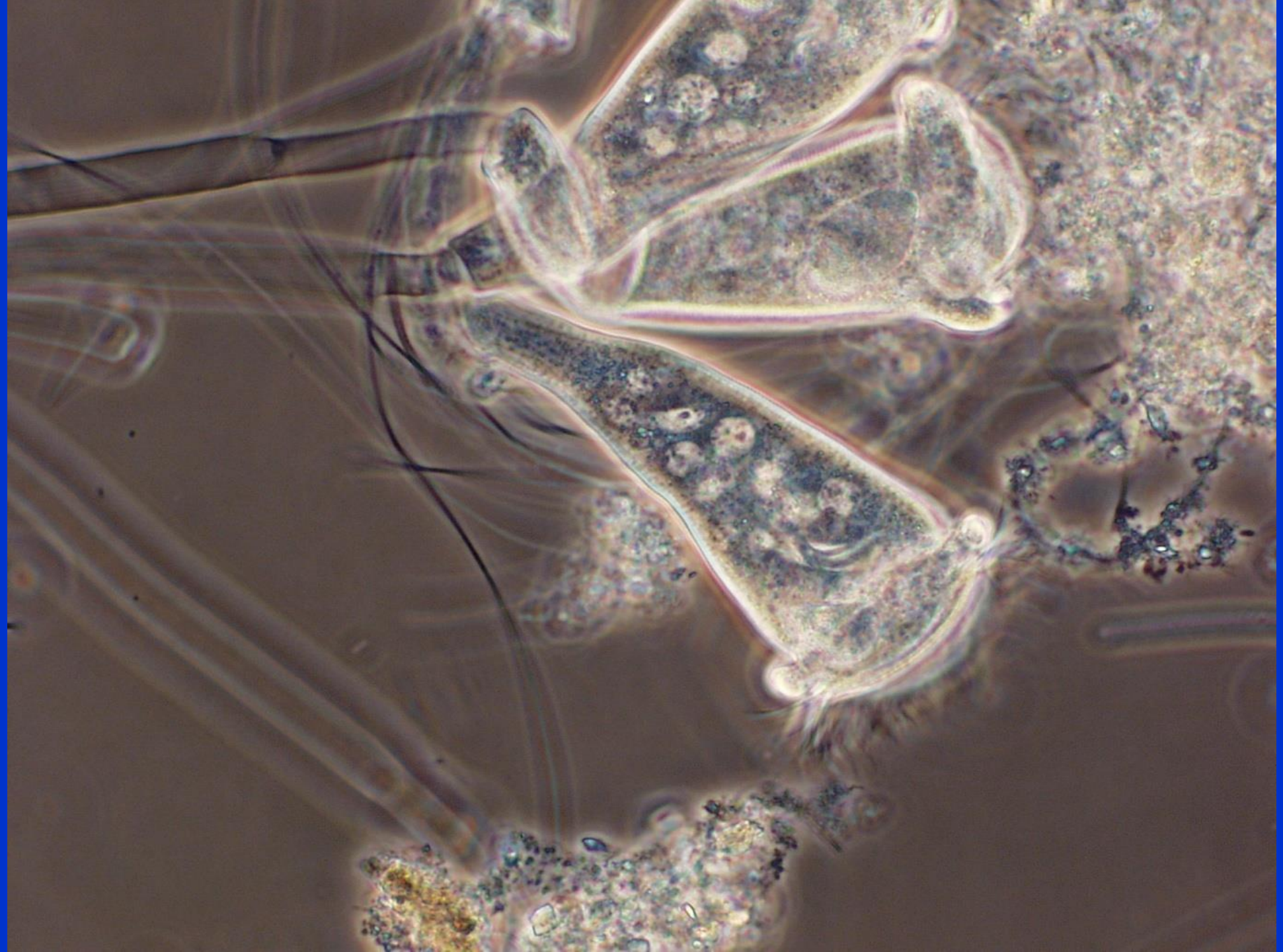
CH30
A100PL
OLYMPUS








1
2
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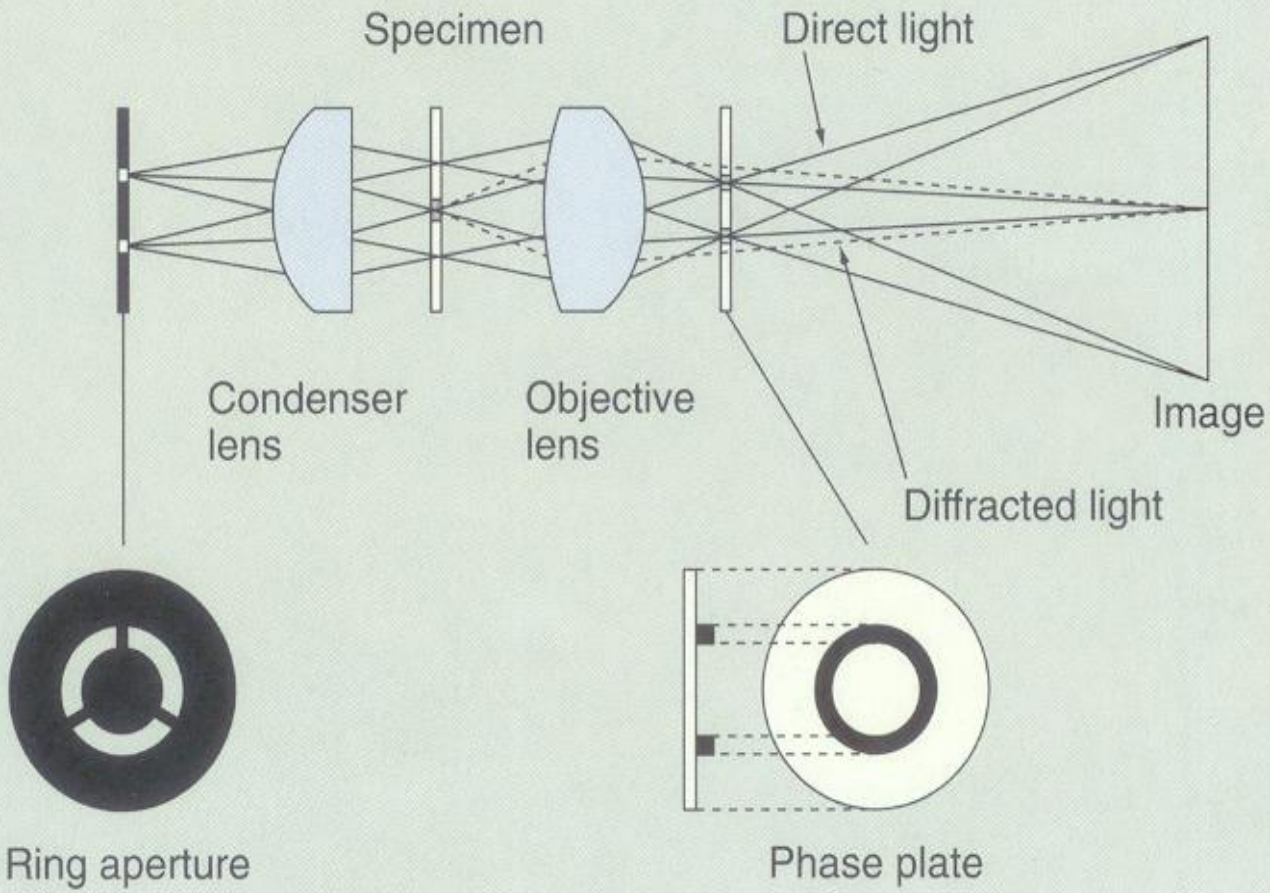
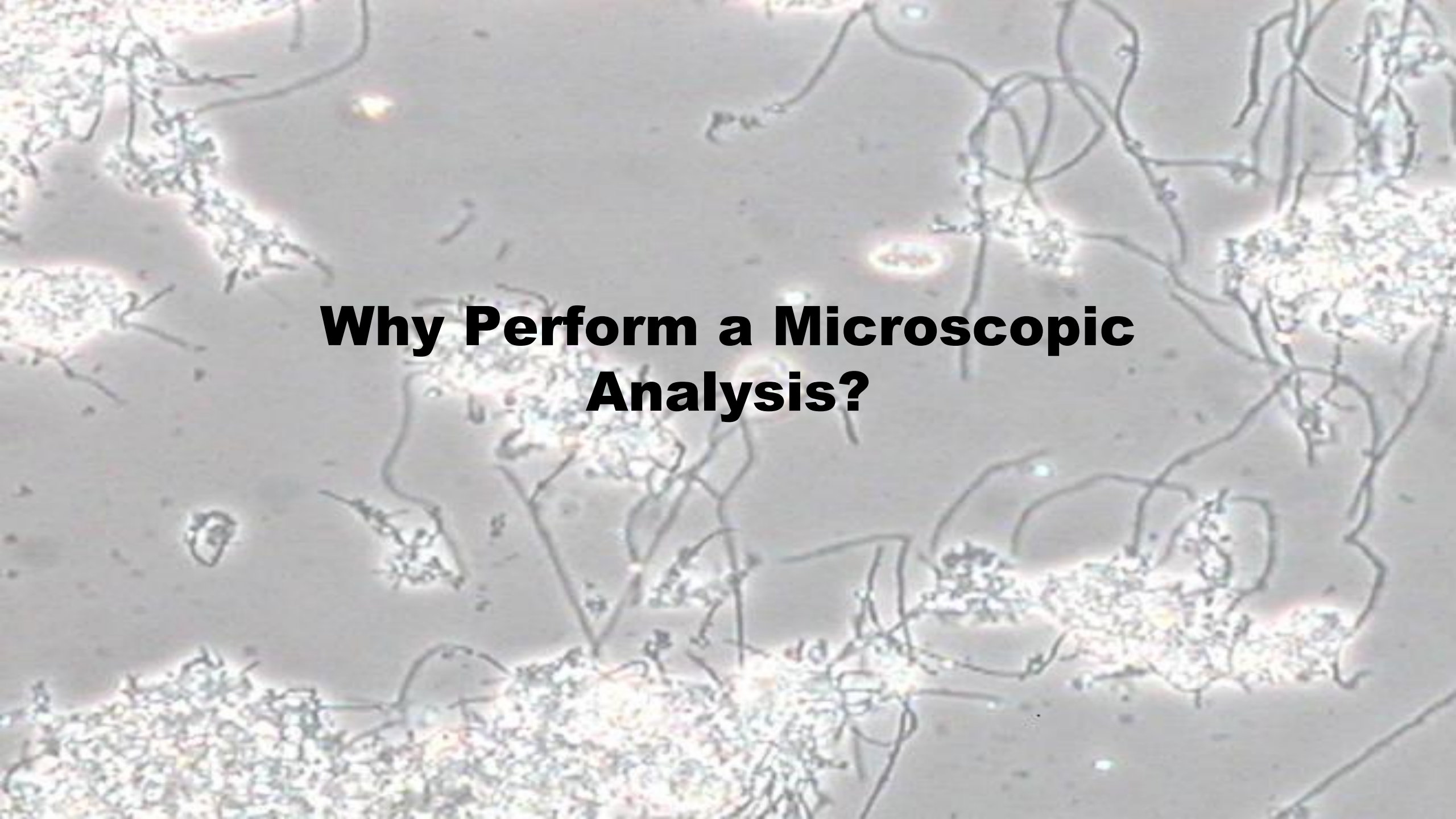
OLYMPUS
CH30
CH30

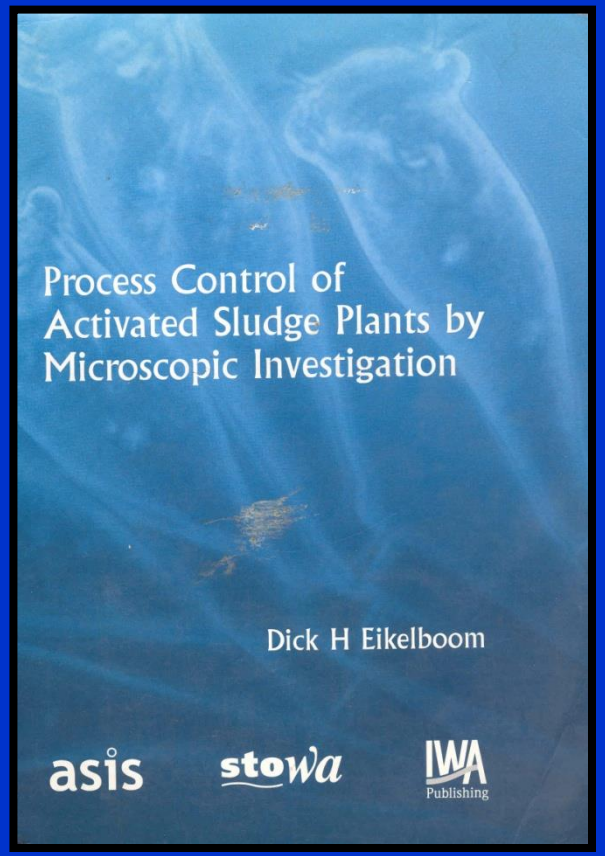
Fig. 1. Phase Contrast Microscopy

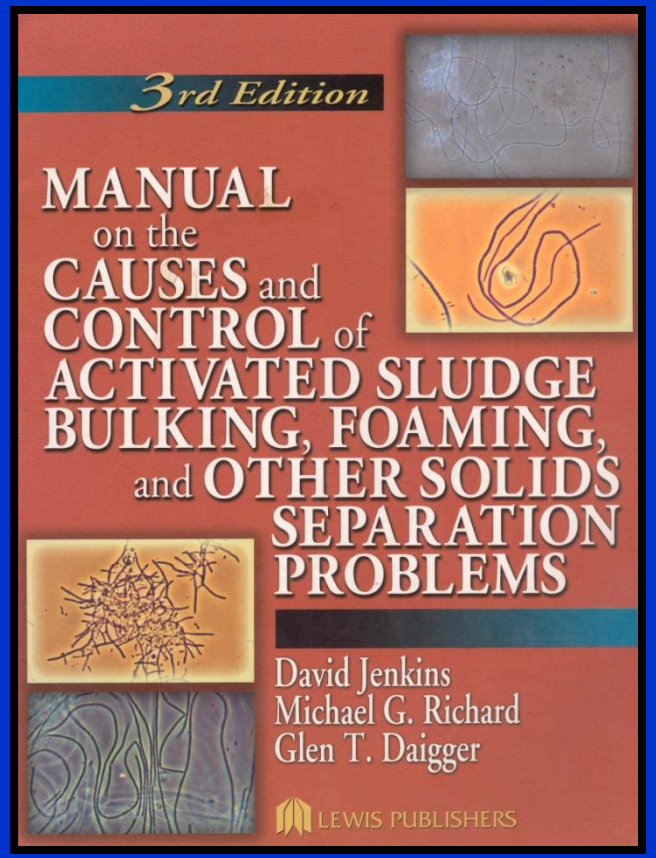


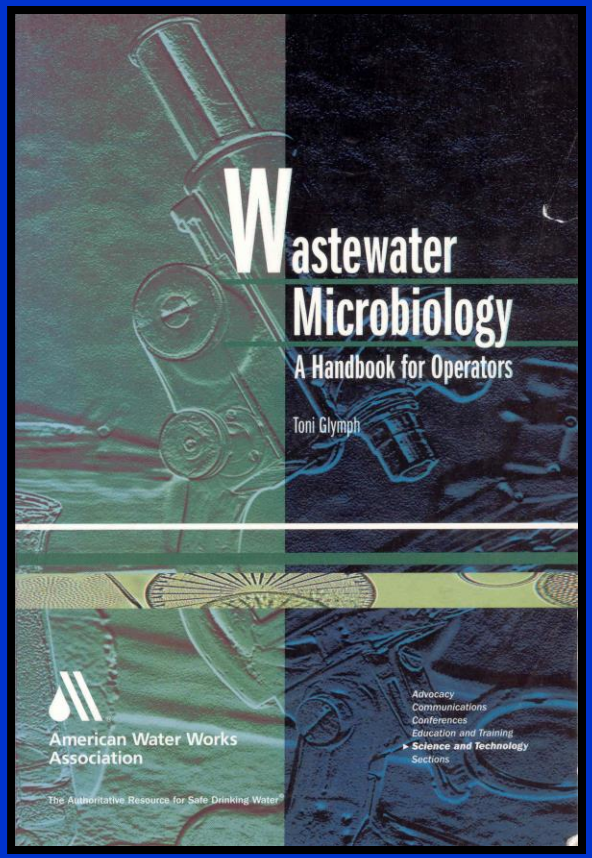


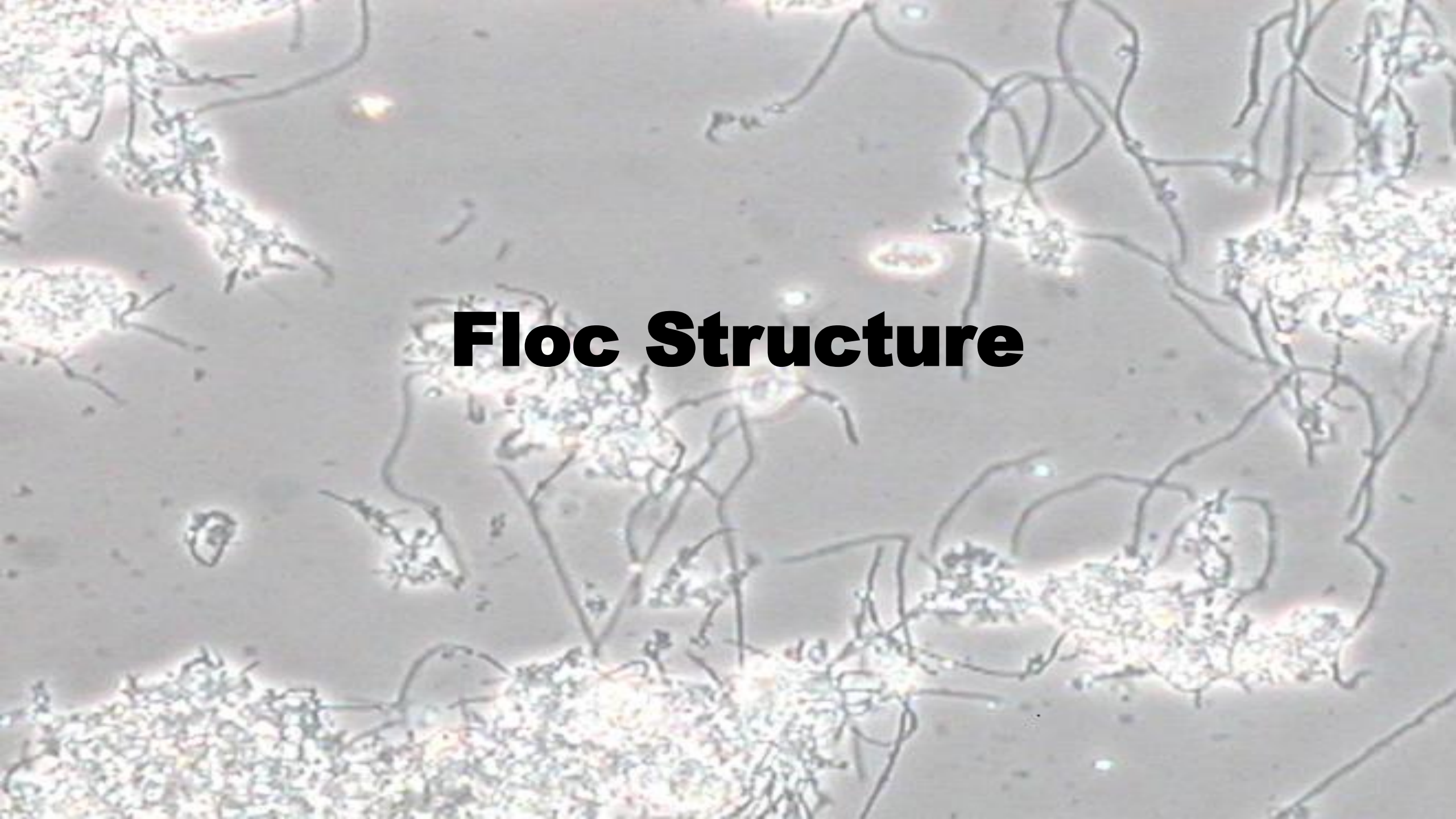
Why Perform a Microscopic Analysis?

Essential Resources



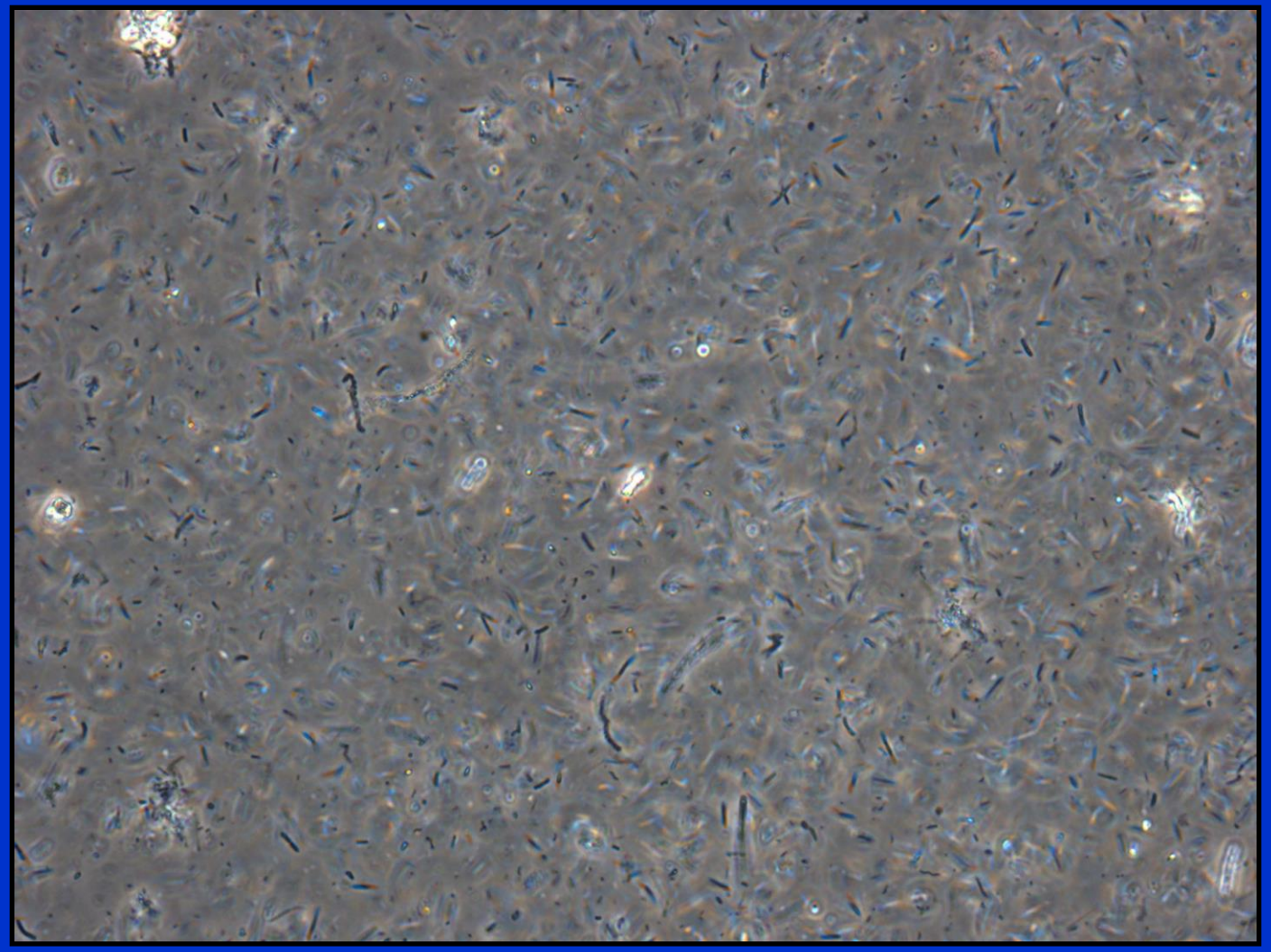






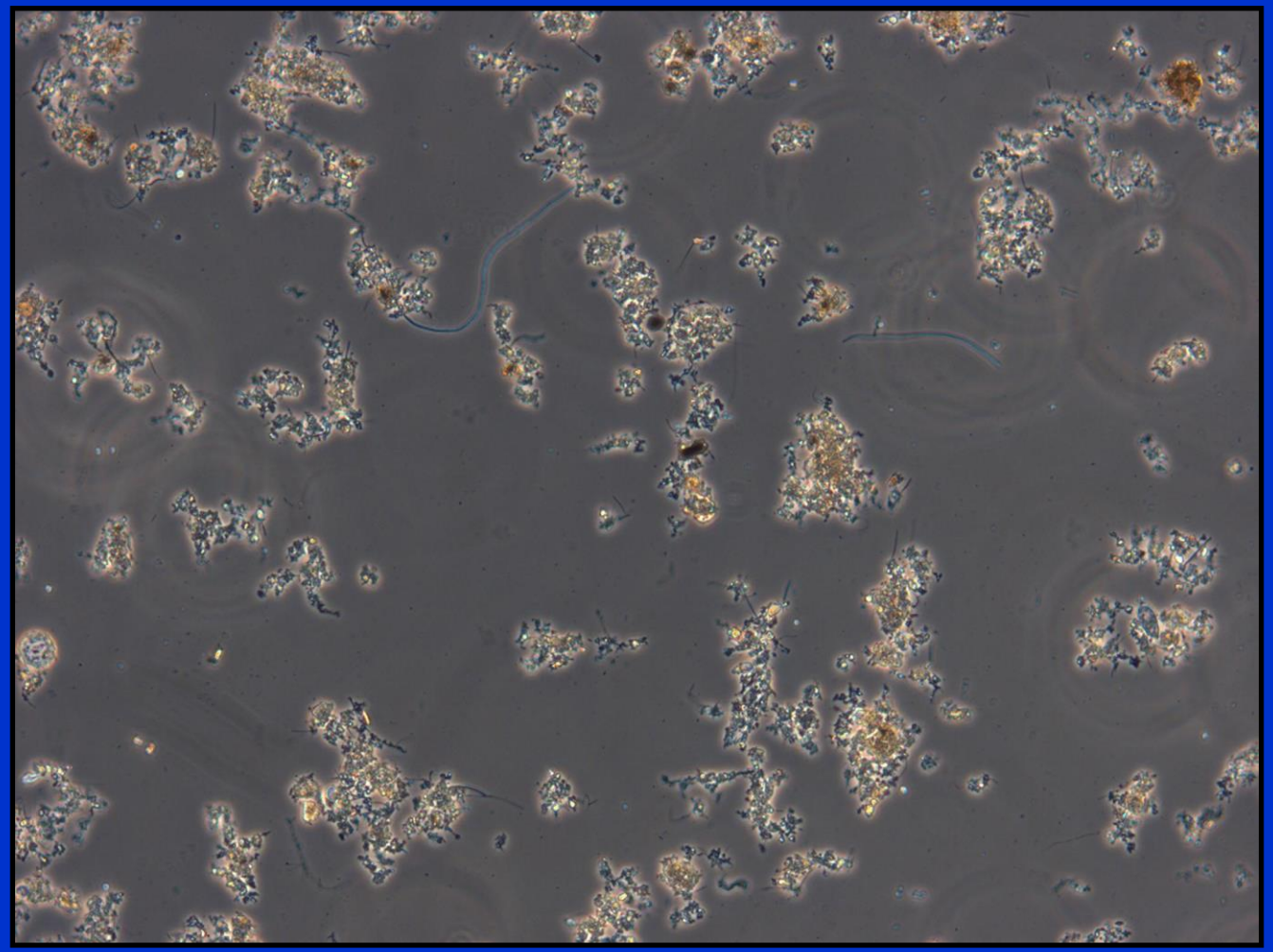
Floc Structure

Dispersed Bacteria

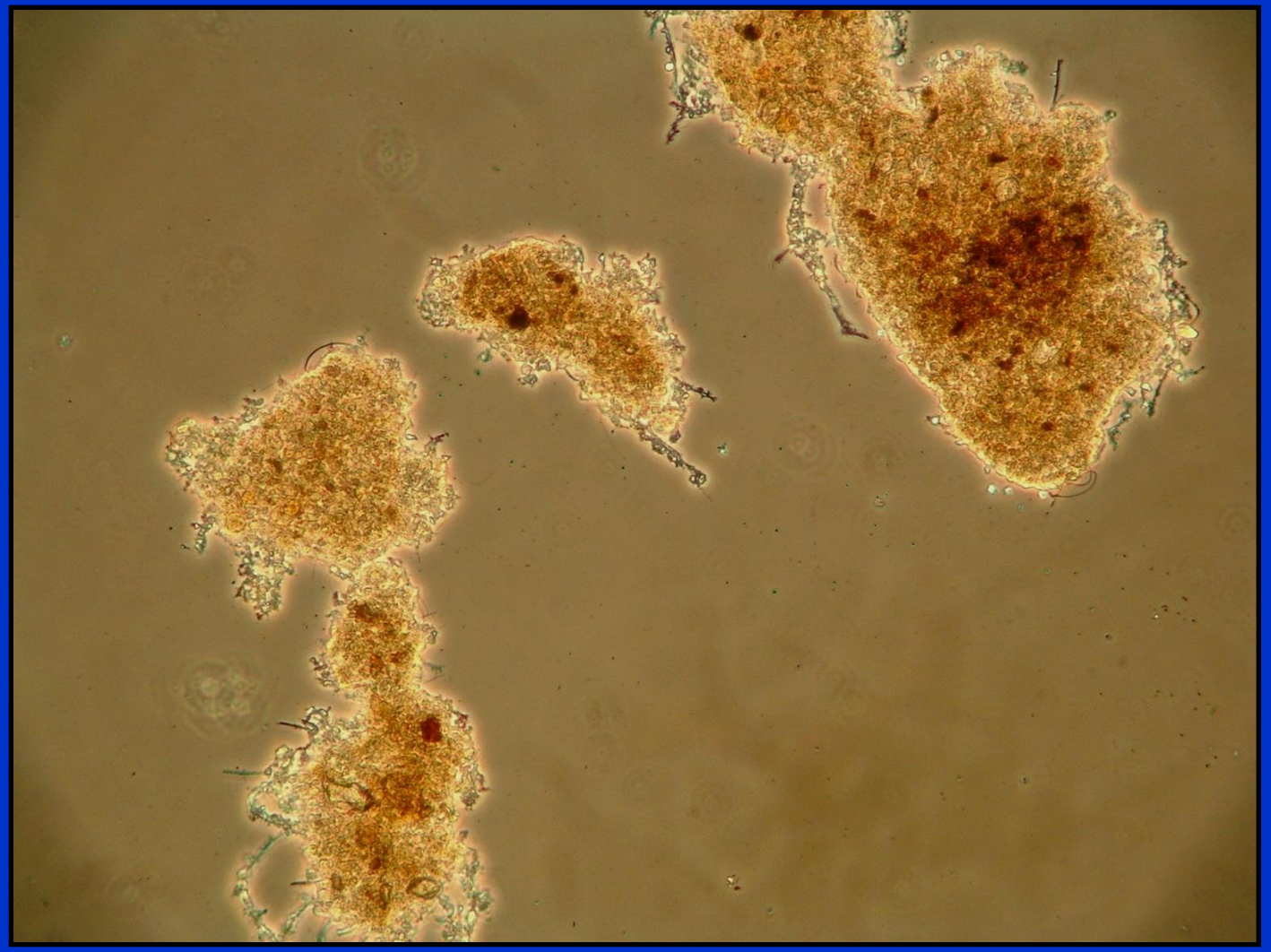


Beginning of Flocculation

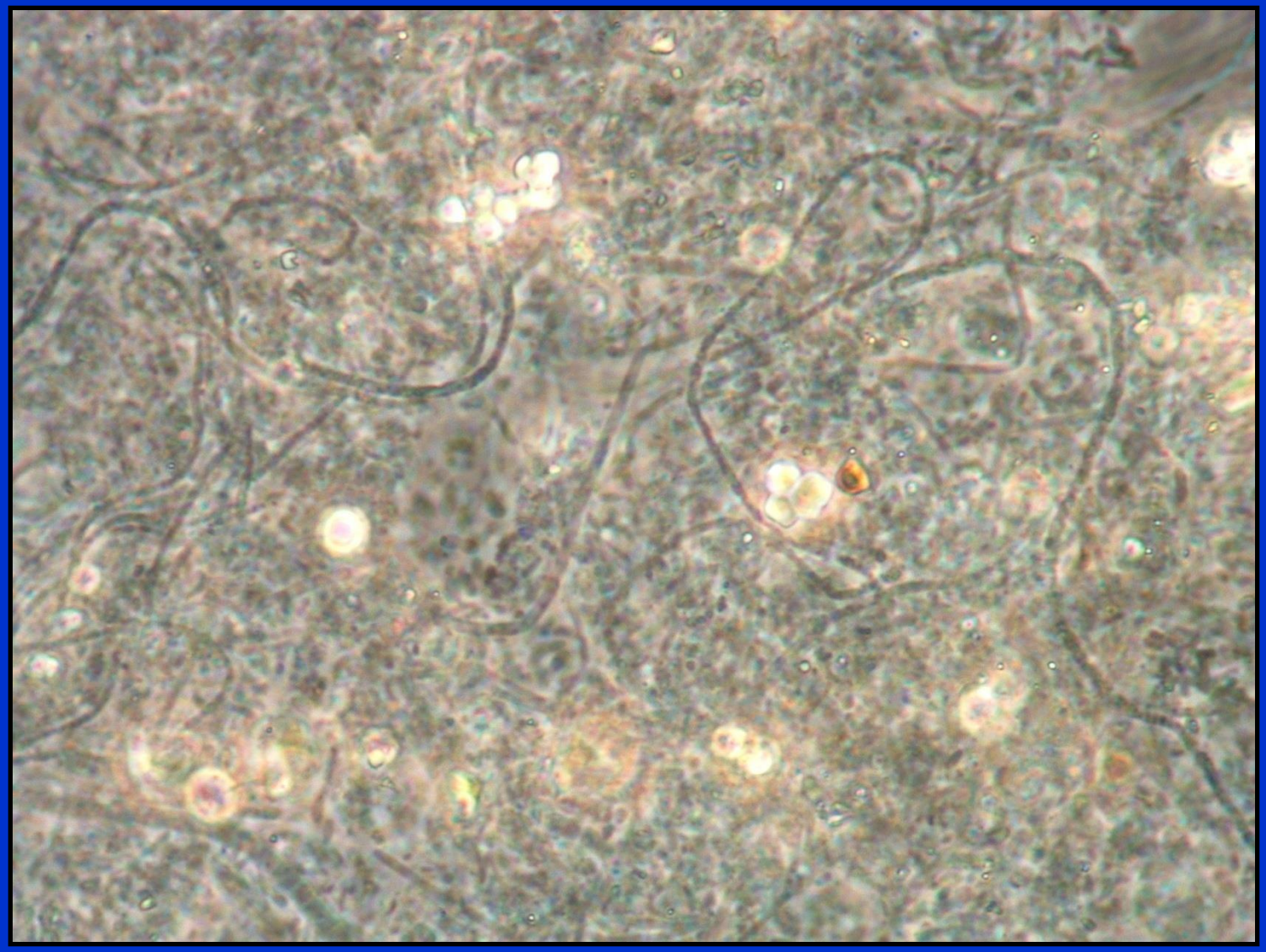
Small Flocs



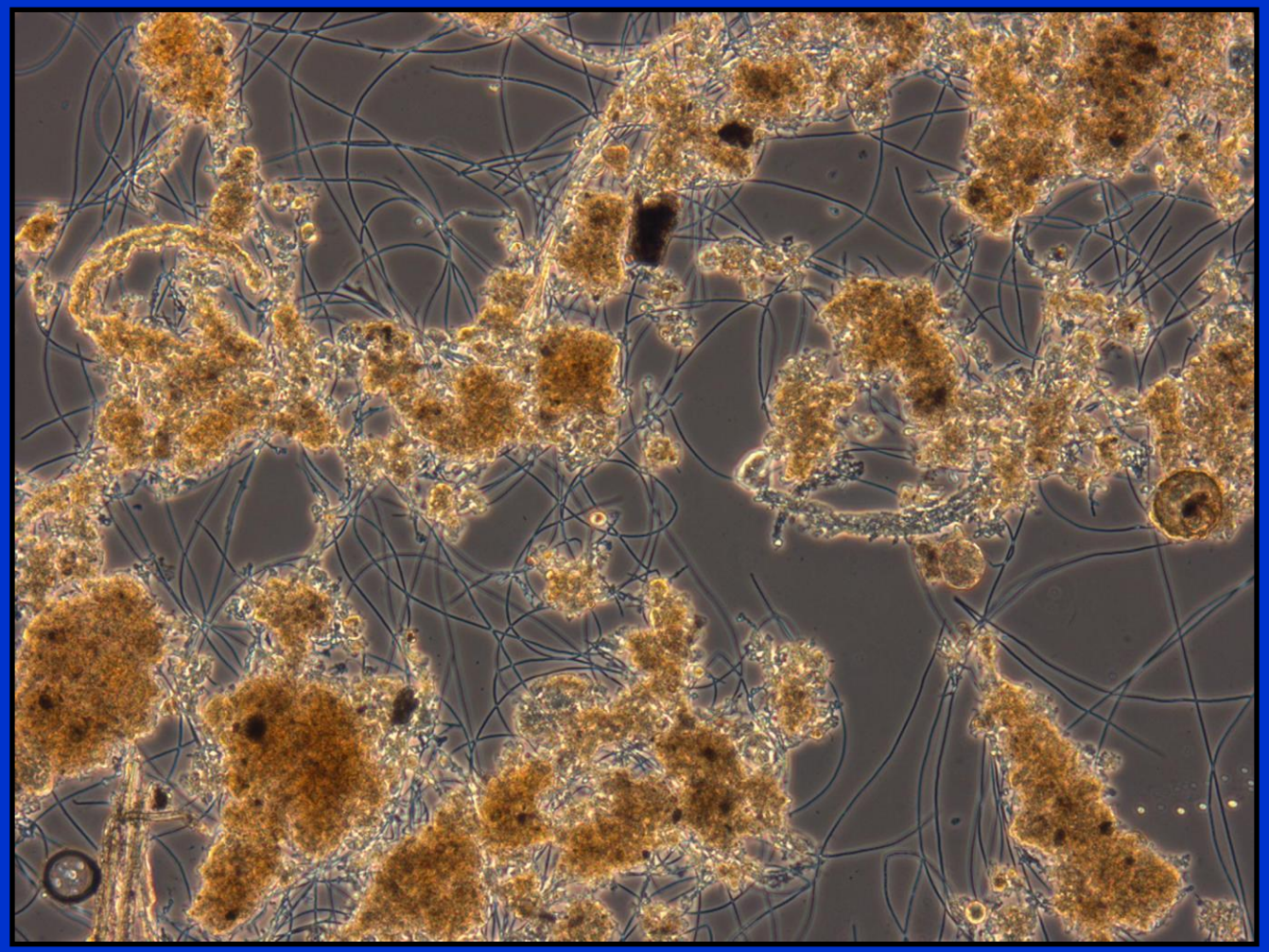
Dense, Compact Flocs



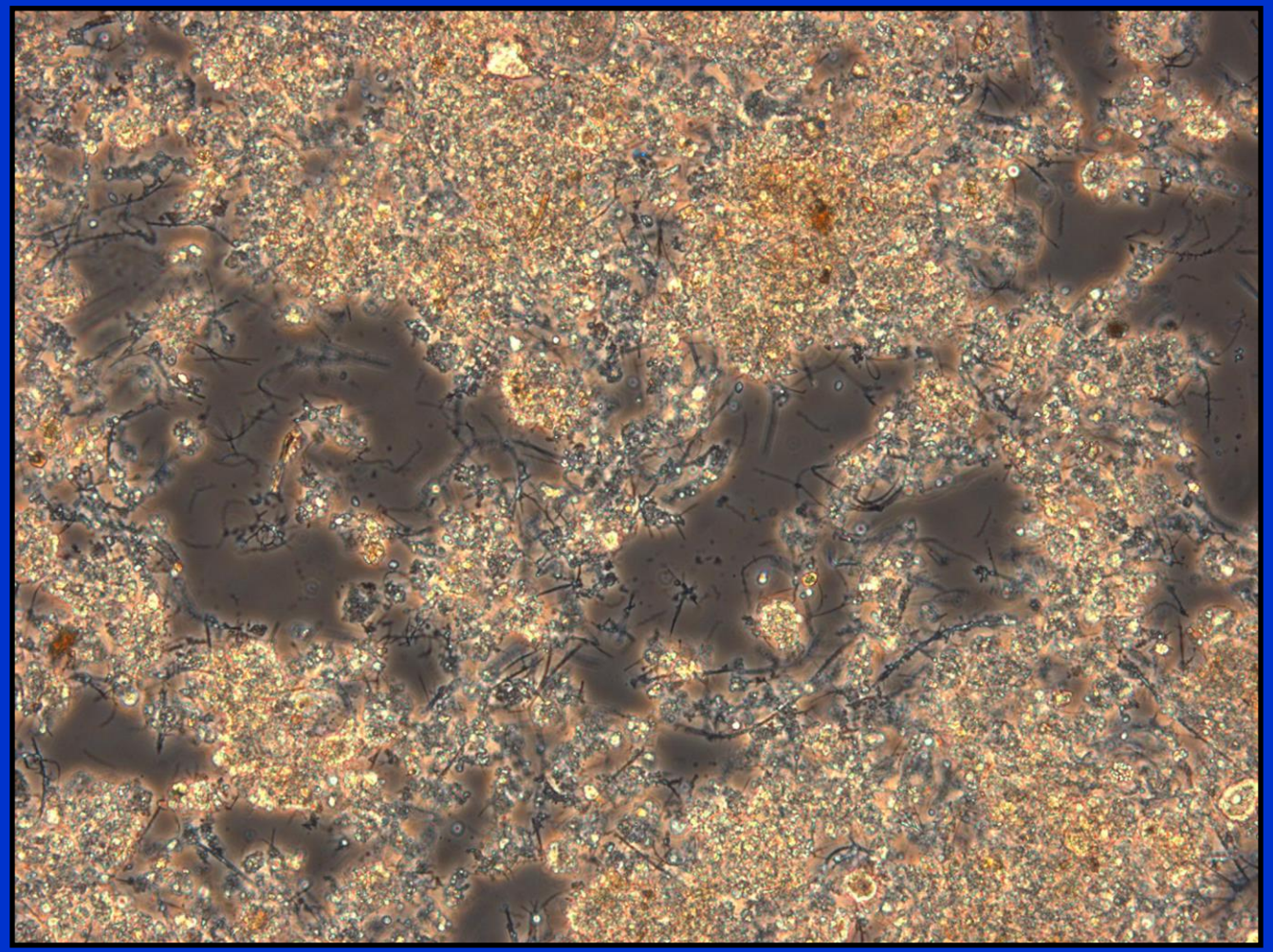
Internal Filaments
Low Density Flocs



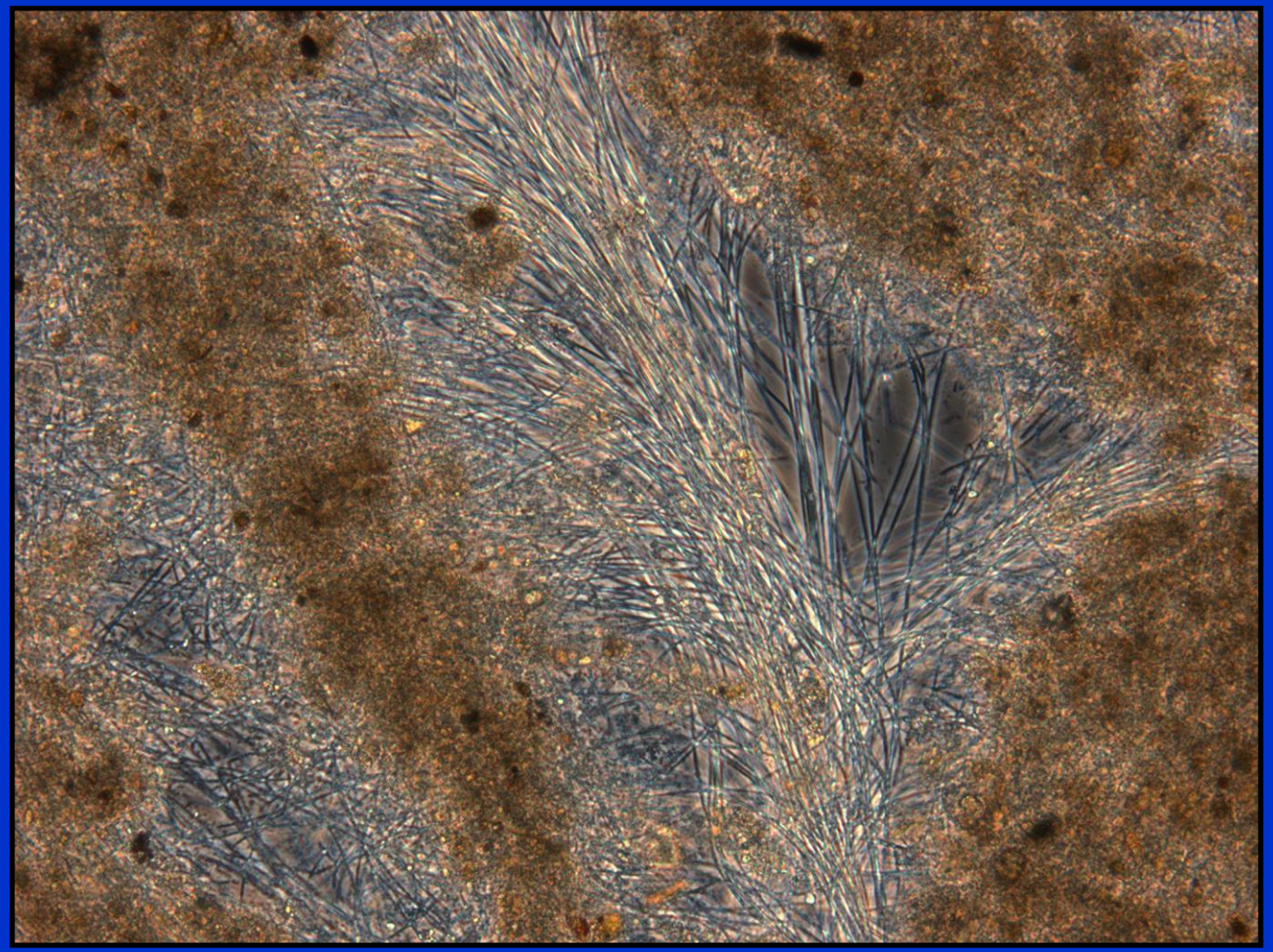
Extending Filaments
Interfloc Bridging

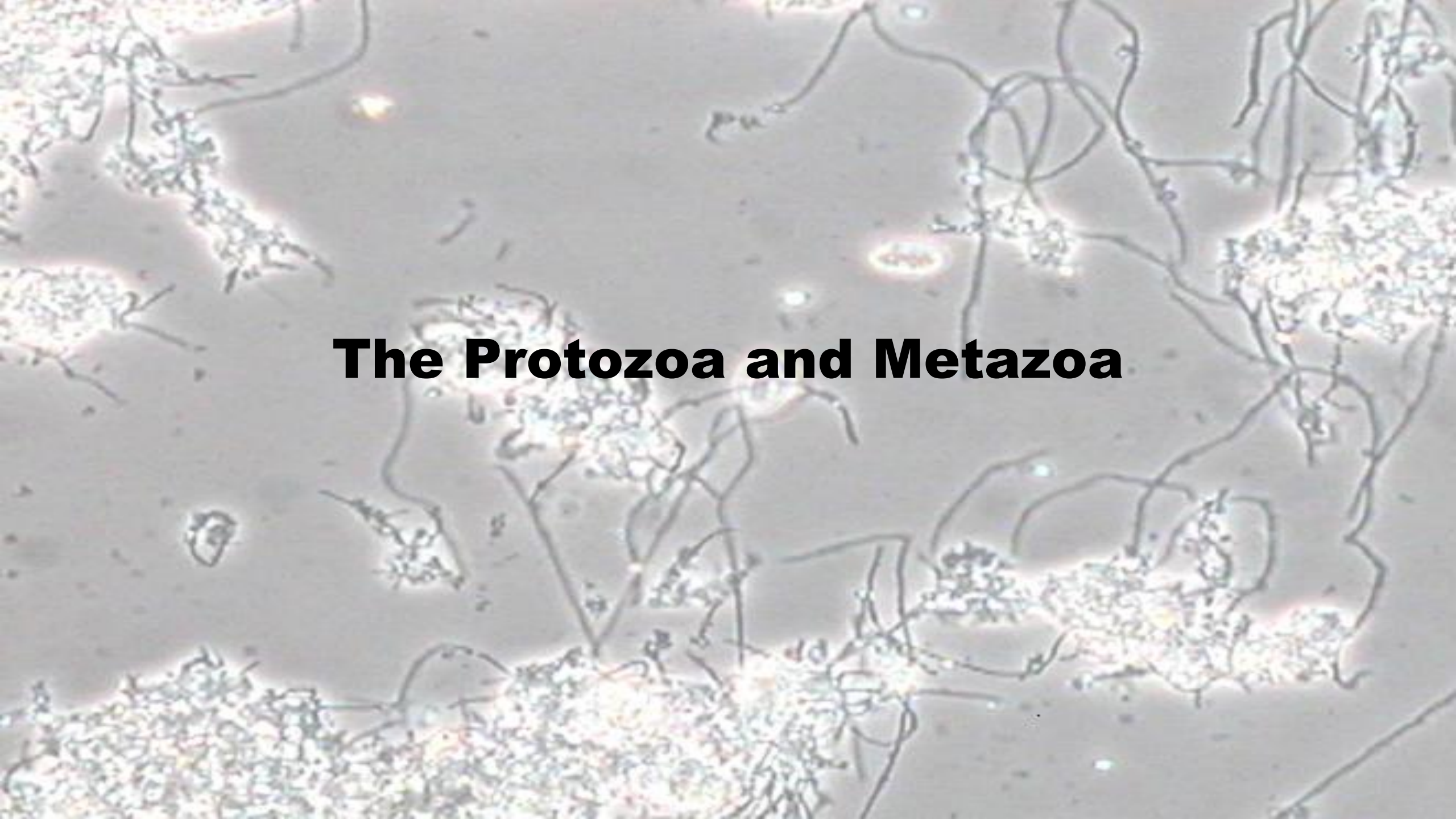


Too Much Mass



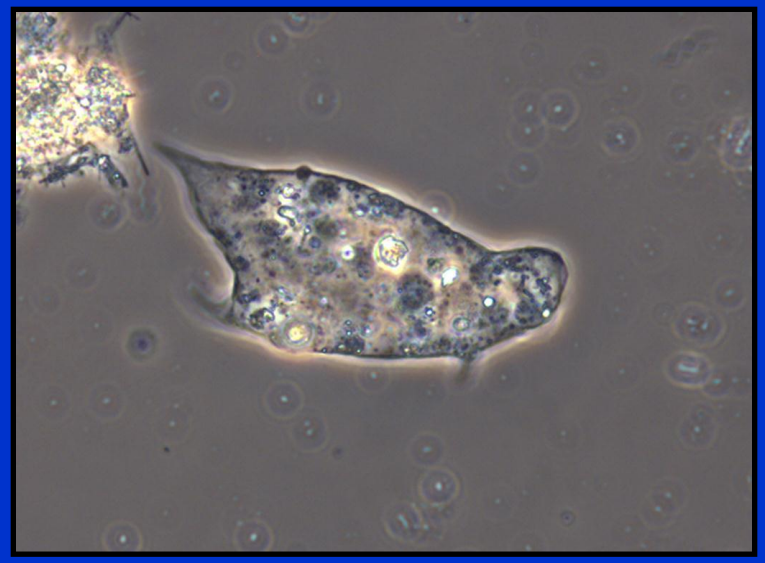
Too Much Filament

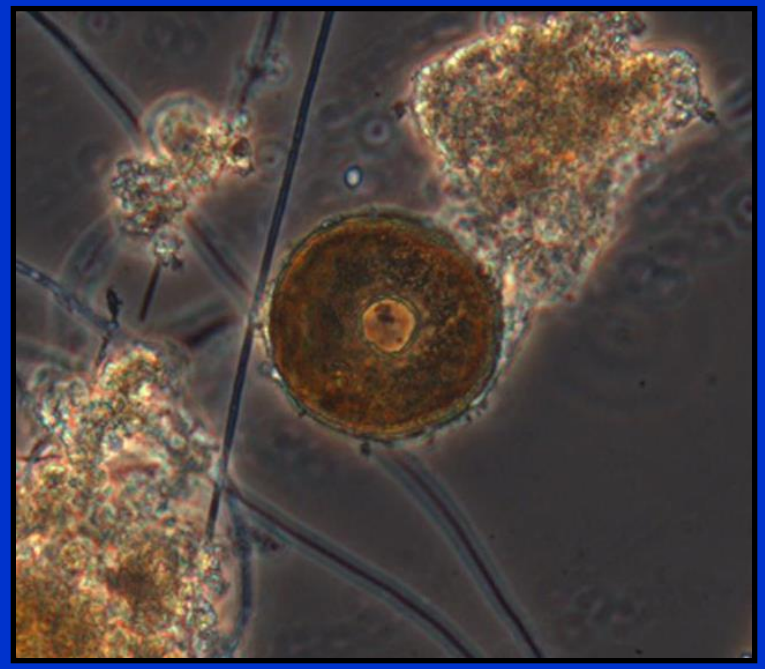


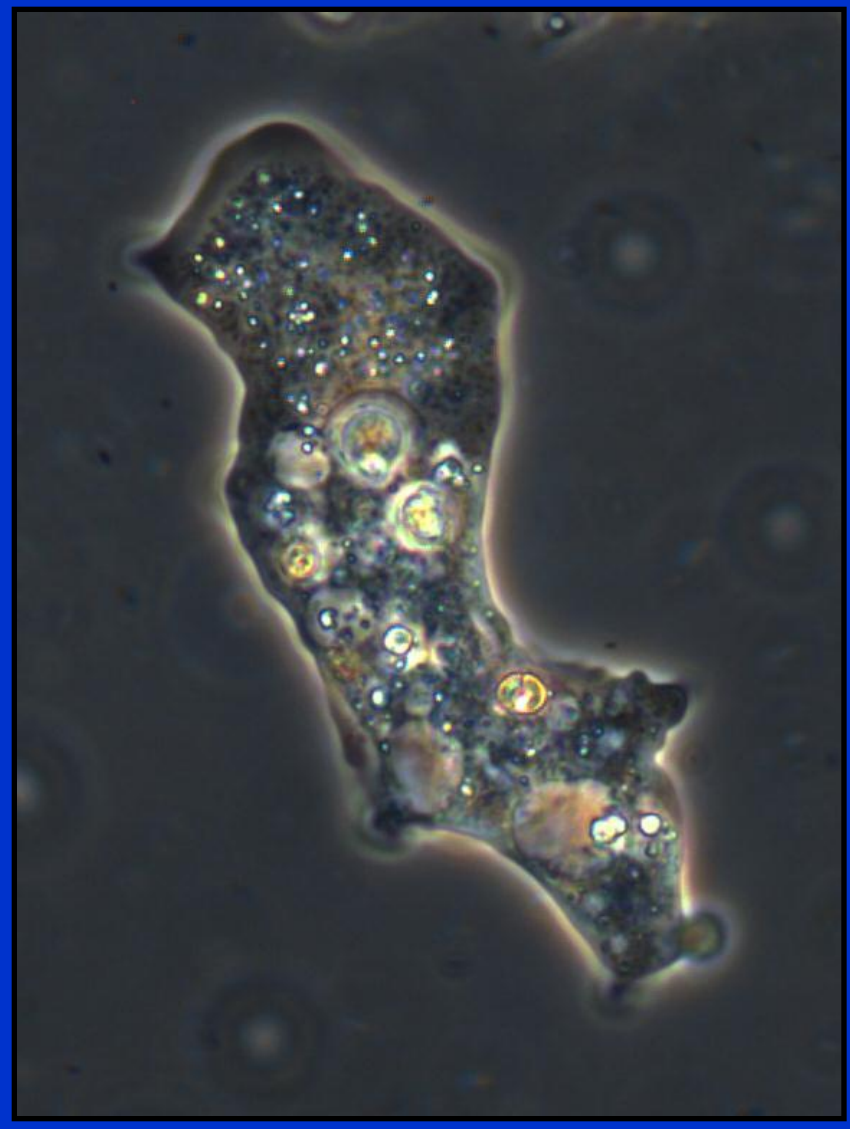


The Protozoa and Metazoa

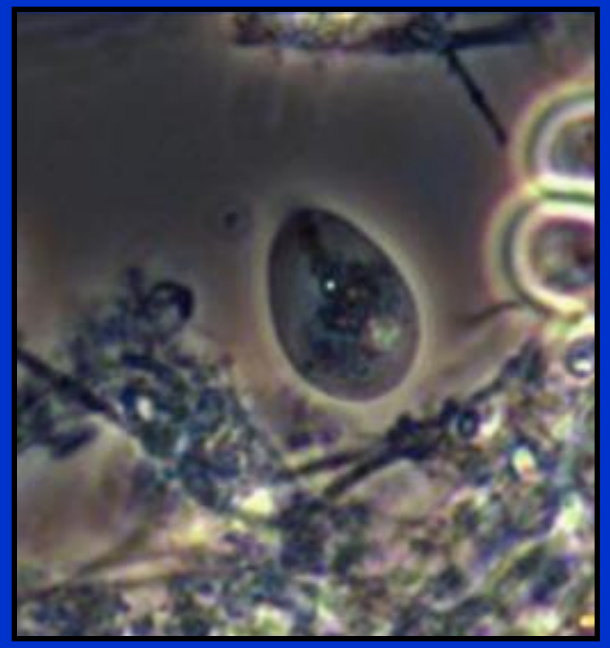
Amoeba

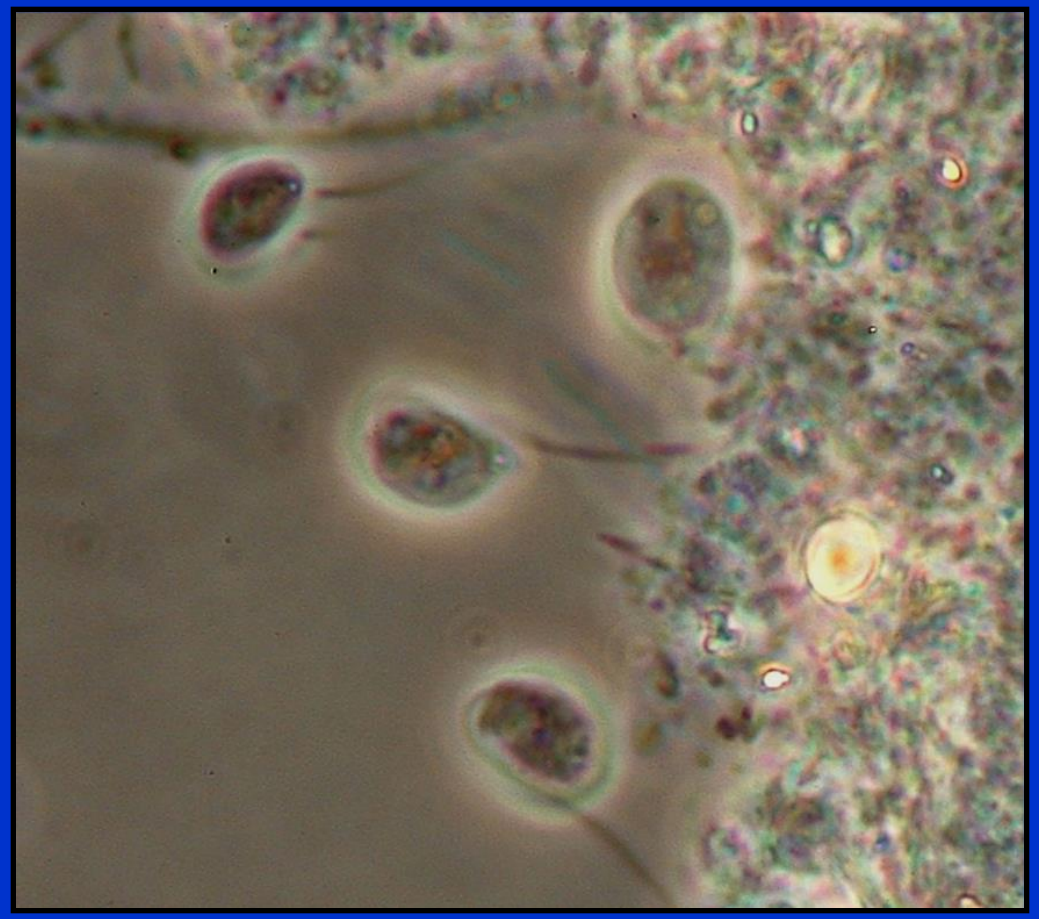




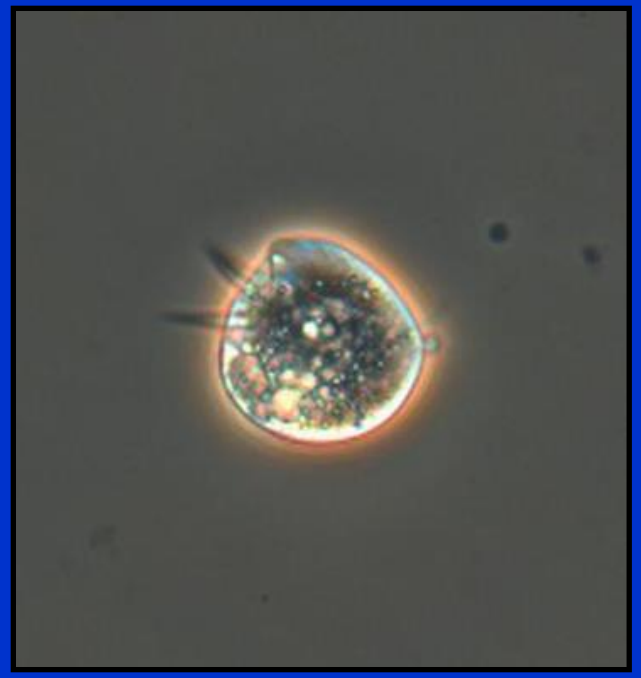


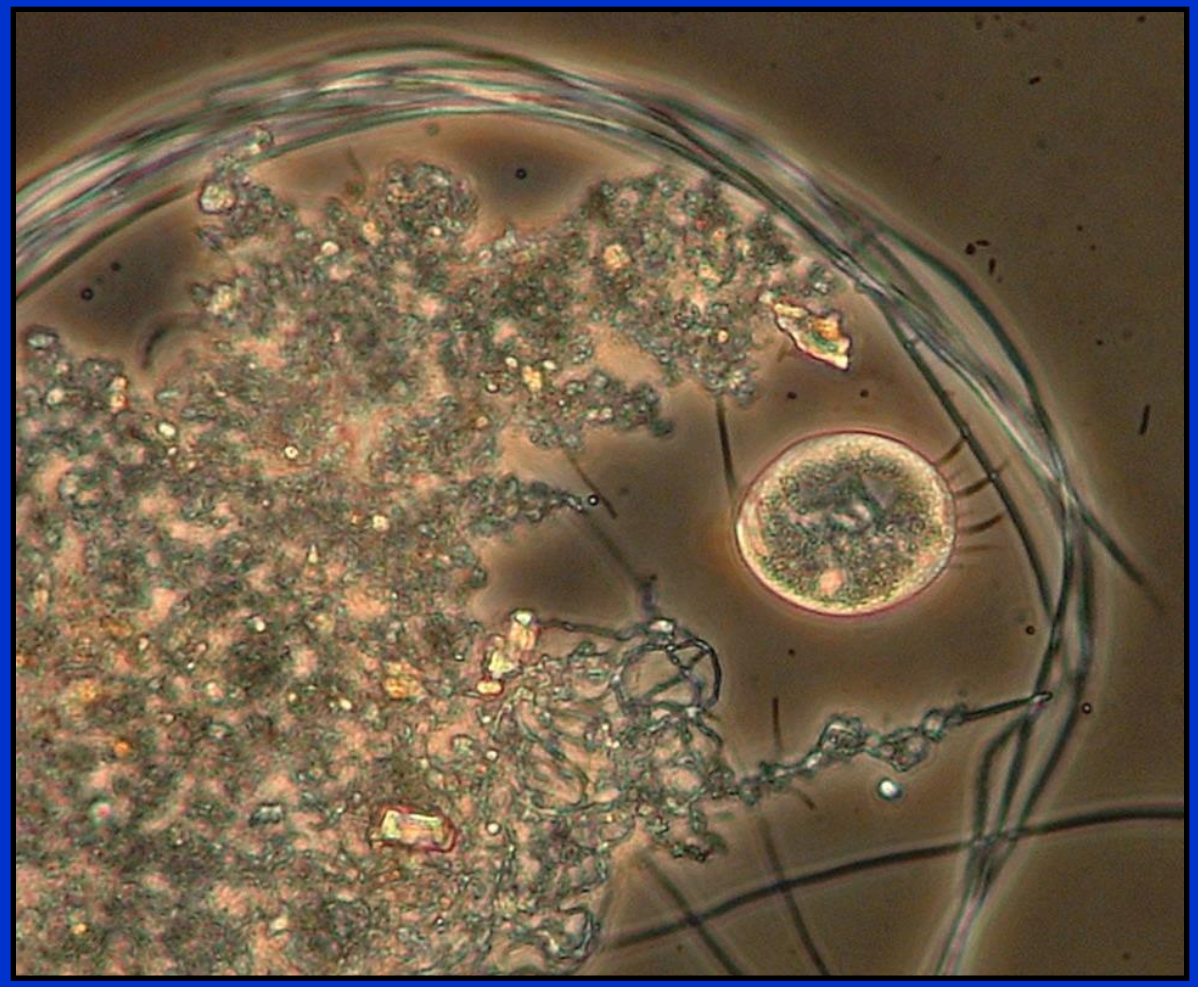
Flagellates



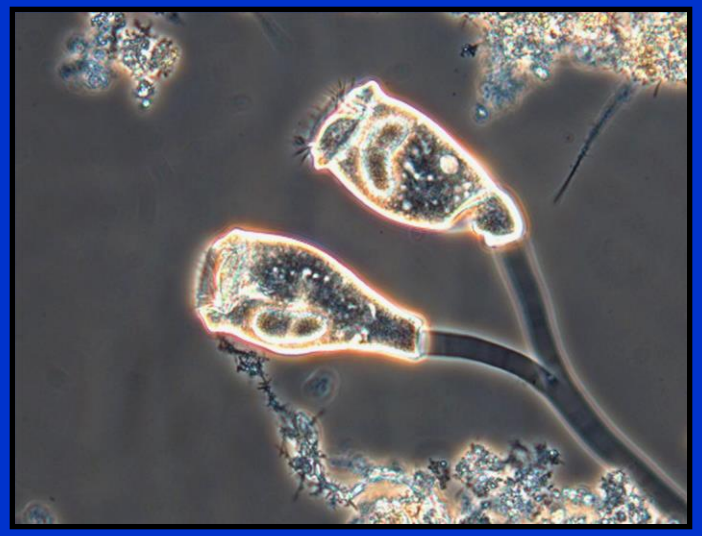


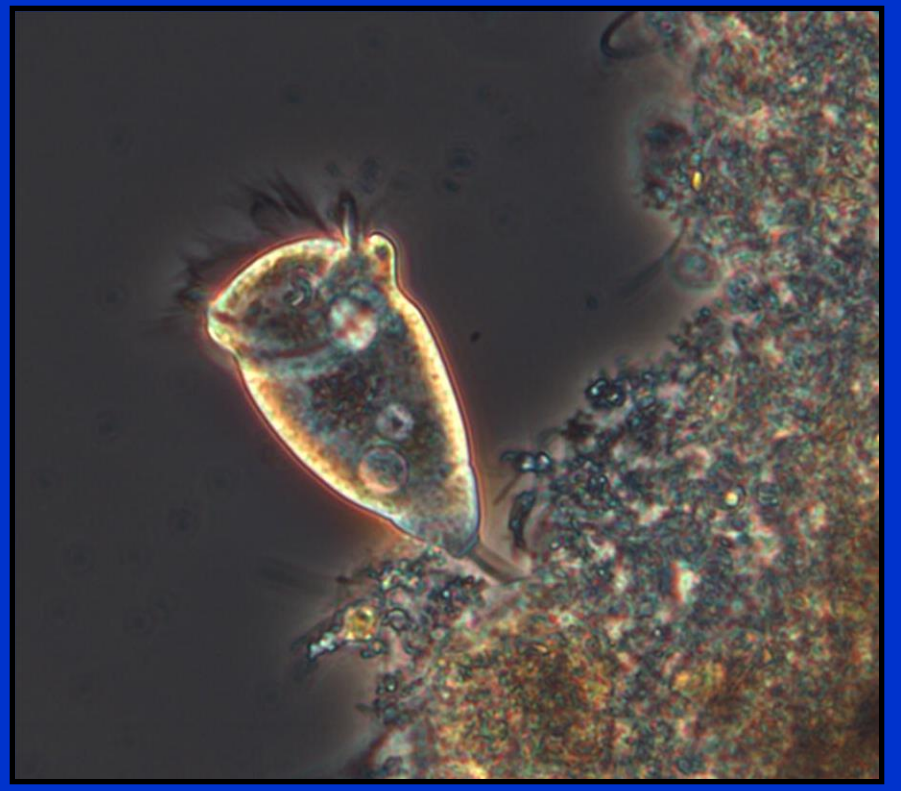
Crawling Ciliates



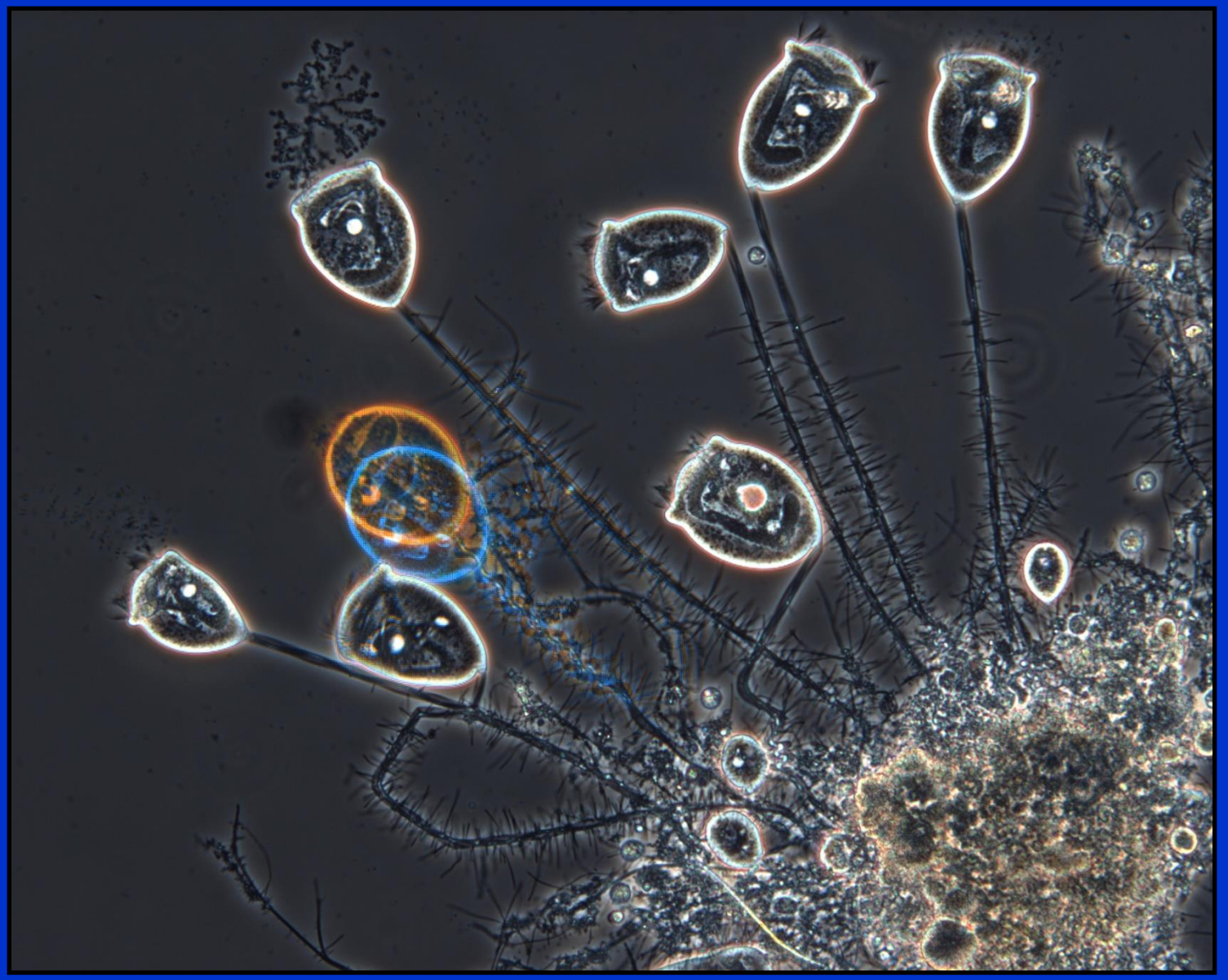


Stalked Ciliates

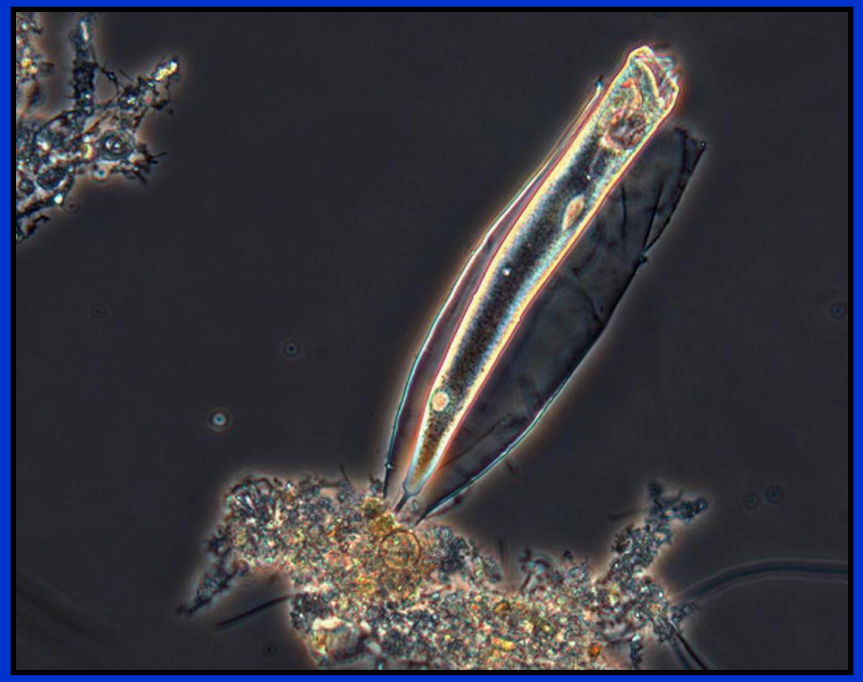


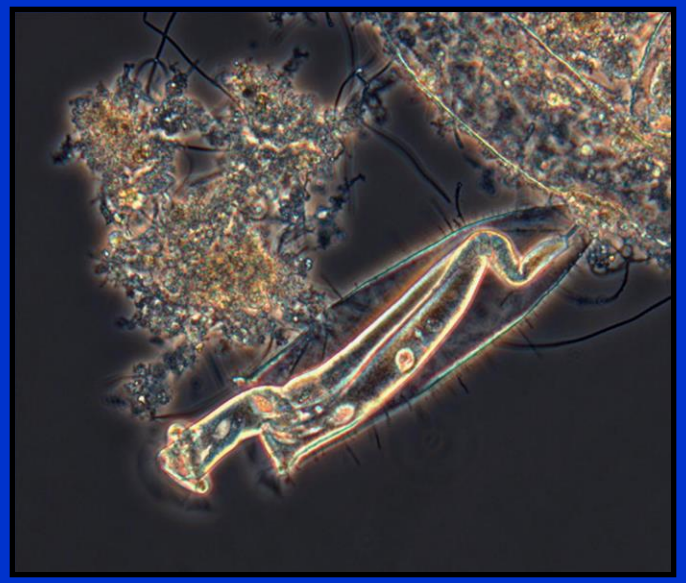


Stalked Ciliates

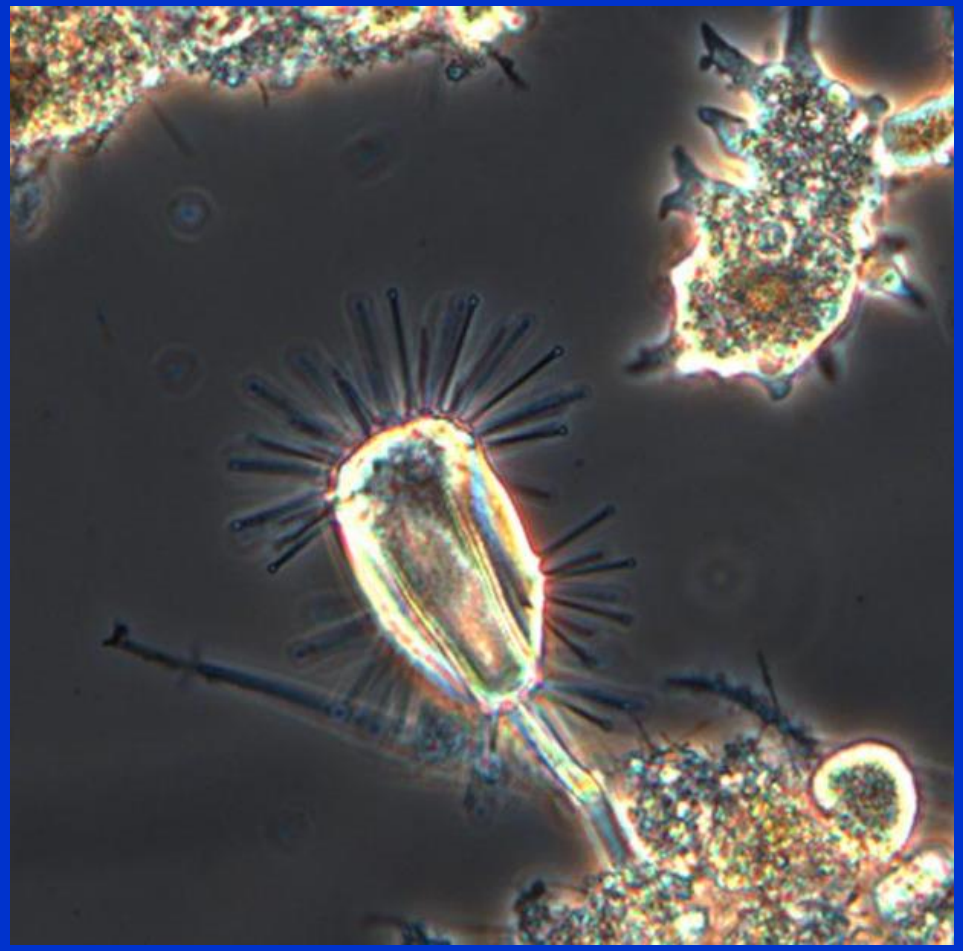


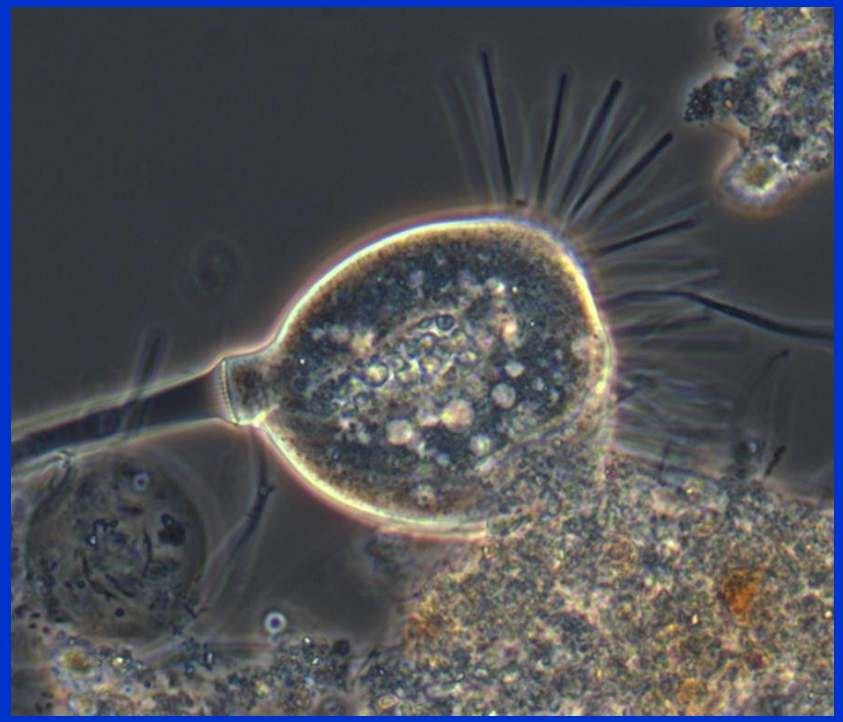
Vaginicola



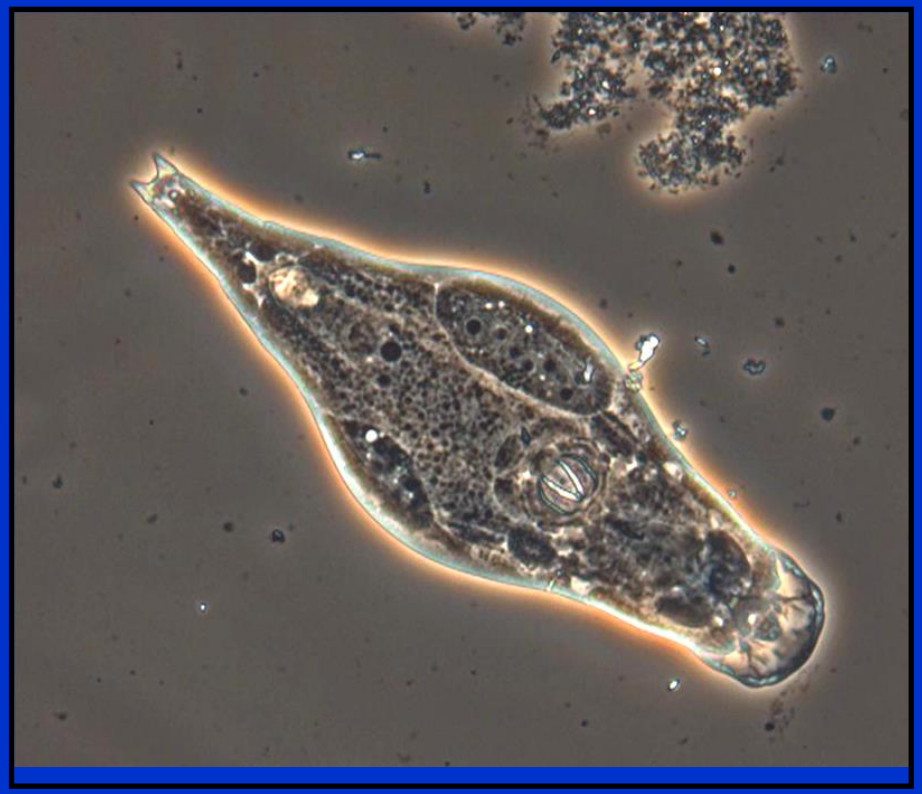


Suctoria



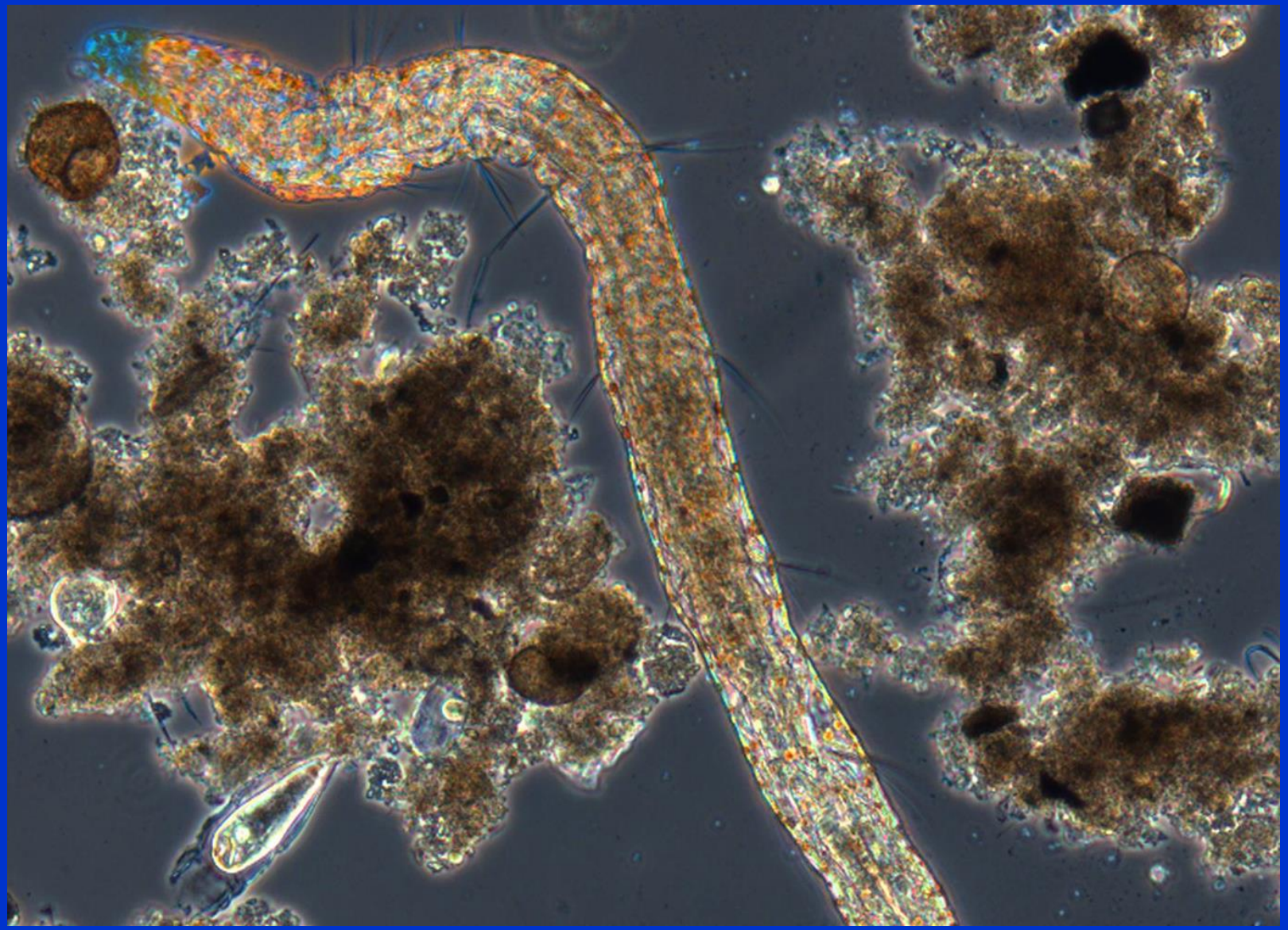


Rotifers



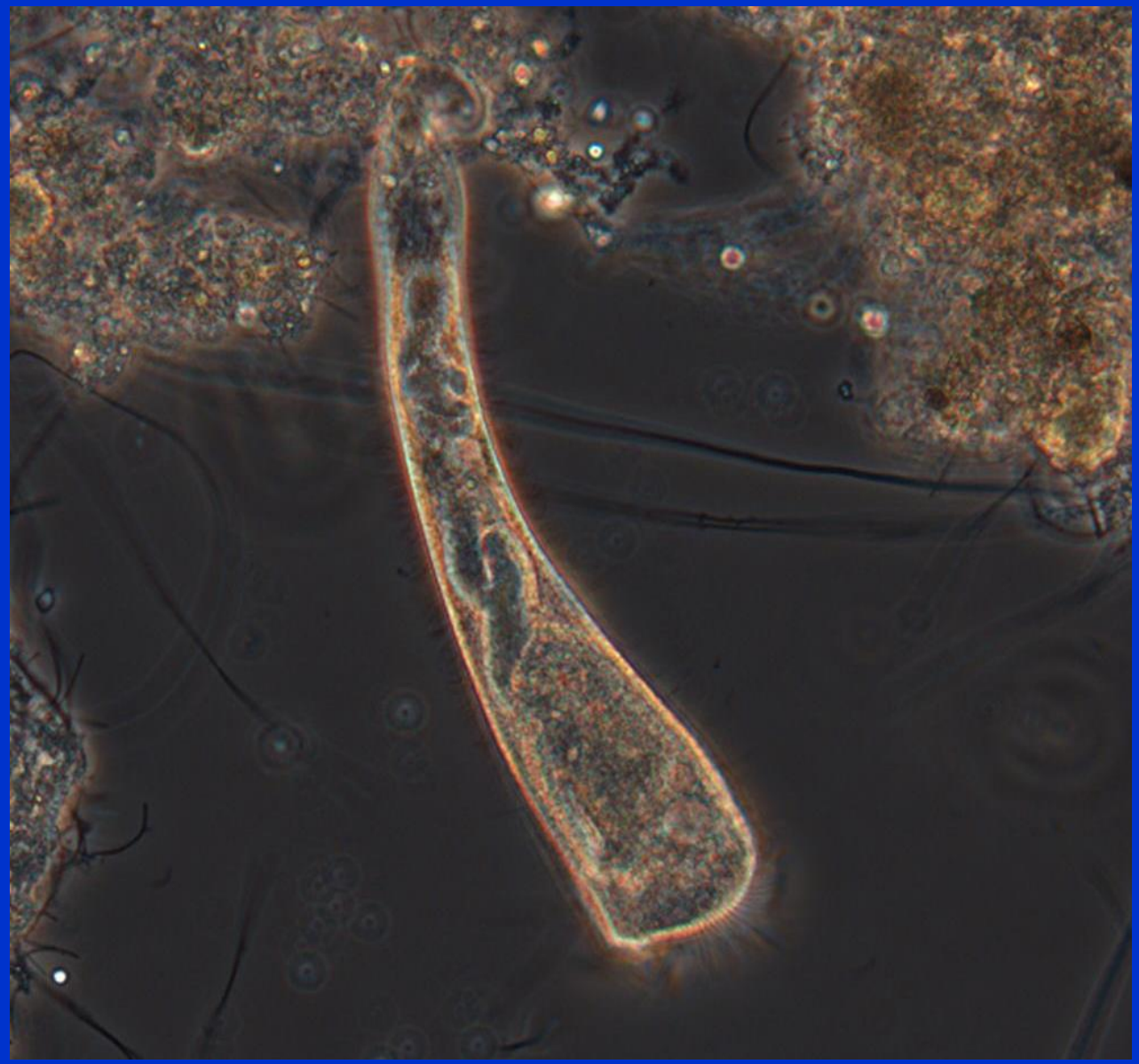
Nematodes

Bristleworm

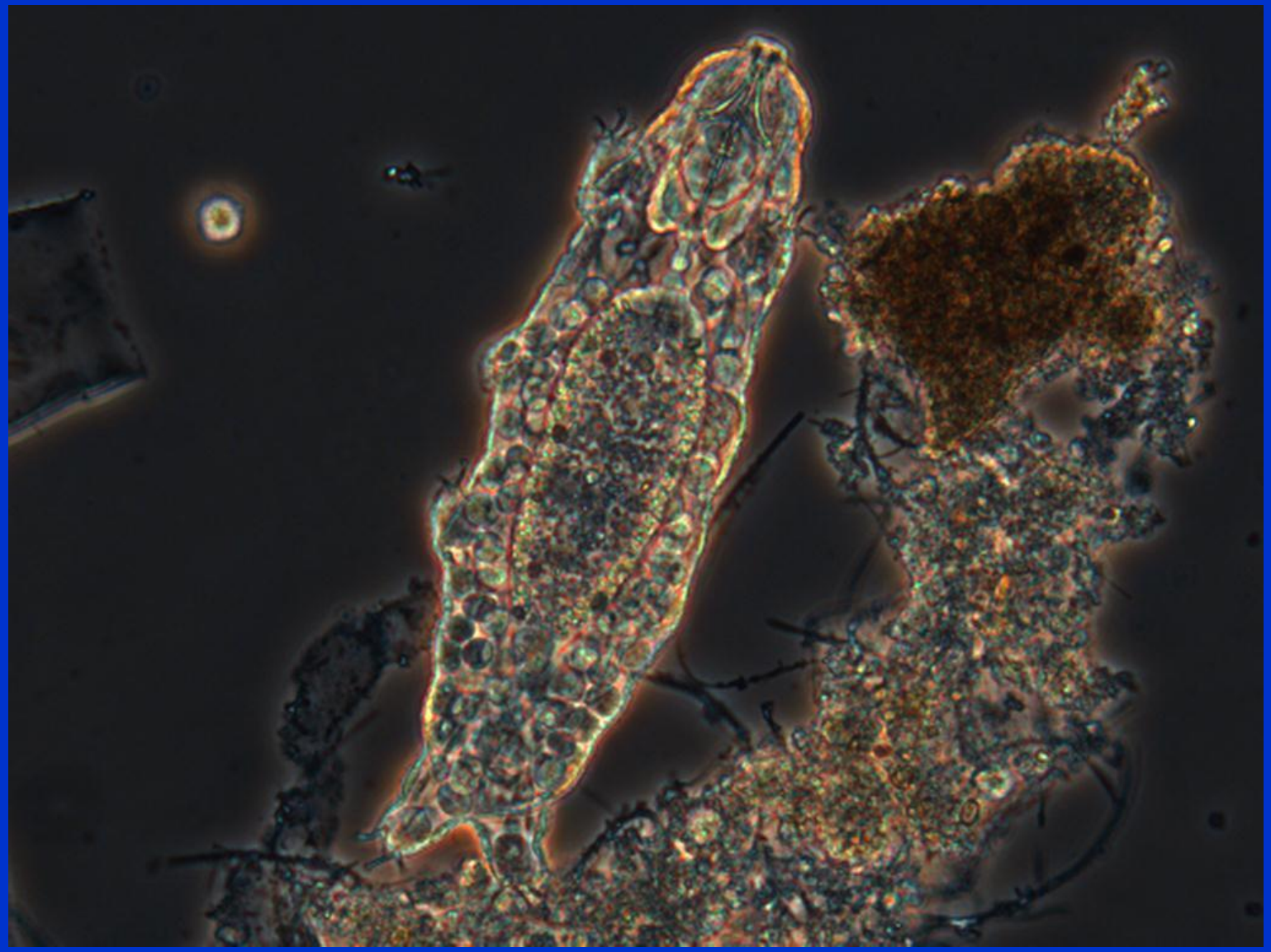


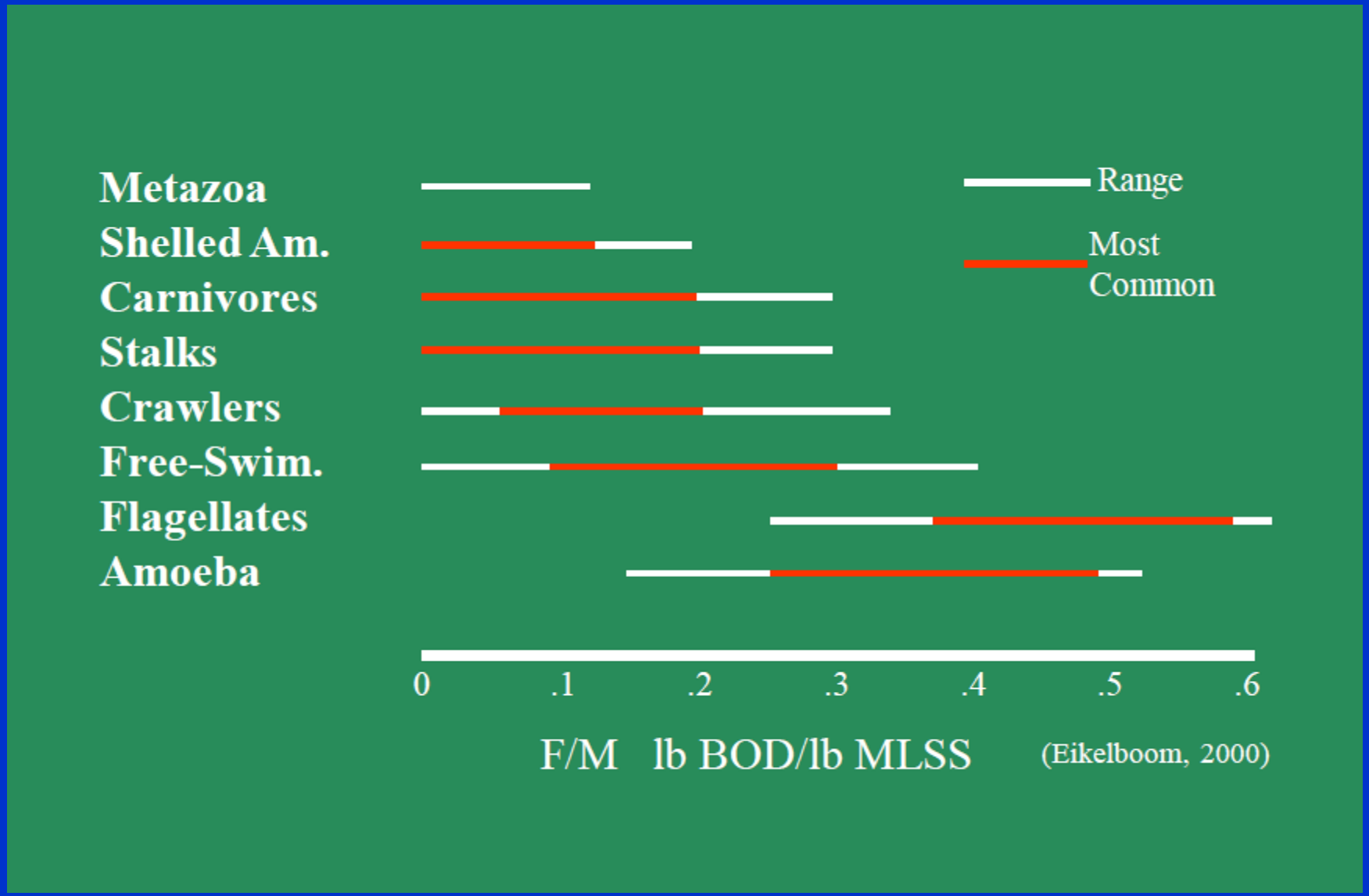
Paramecium

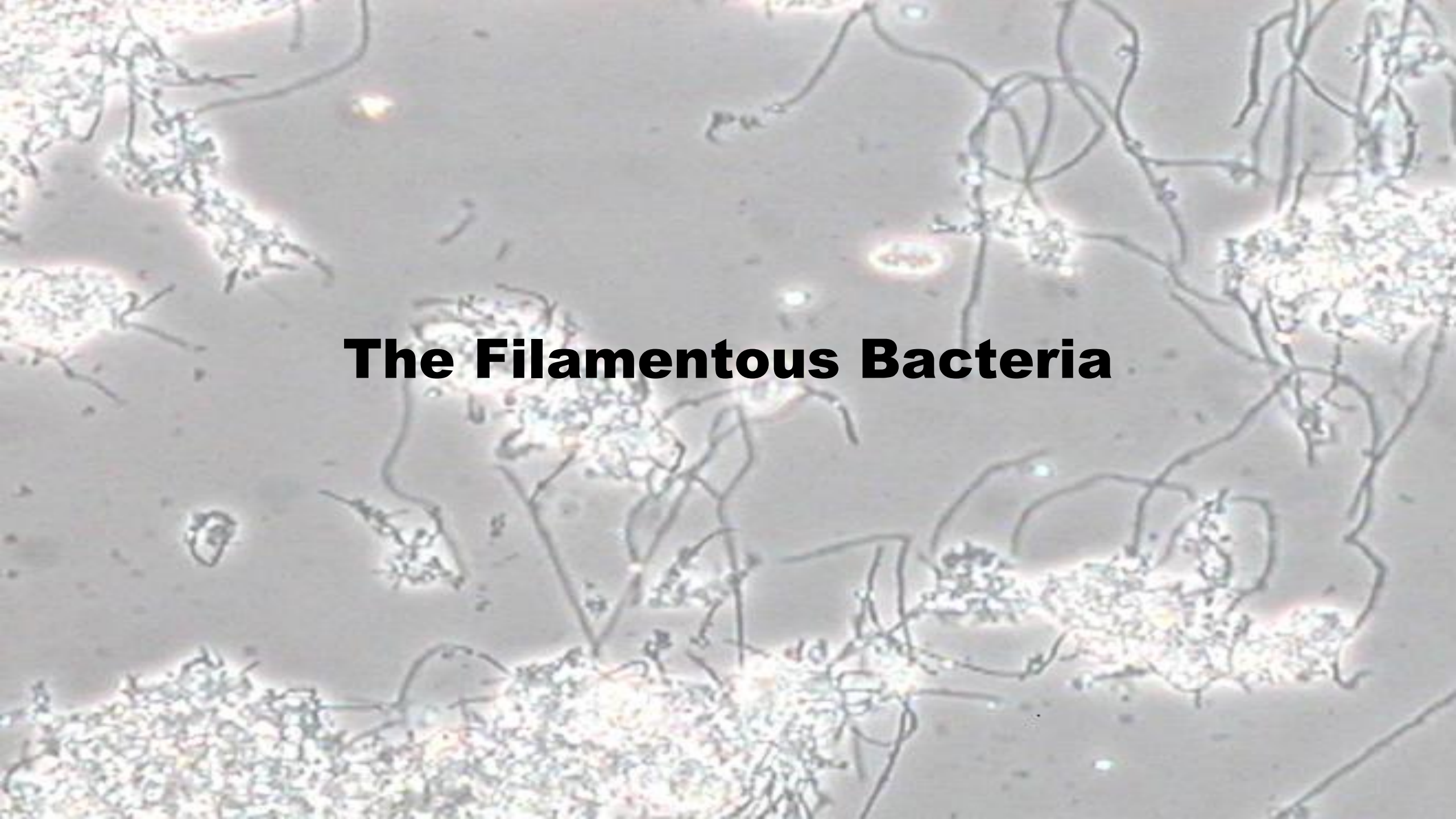
Stentor



Water Bear




Metazoa
Shelled Am.
Carnivores
Stalks
Crawlers
Free-Swim.
Flagellates
Amoeba
Range
Most Common
0
.1
.2
.3
.4
.5
.6
F/M lb BOD/lb MLSS
(Eikelboom, 2000)



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

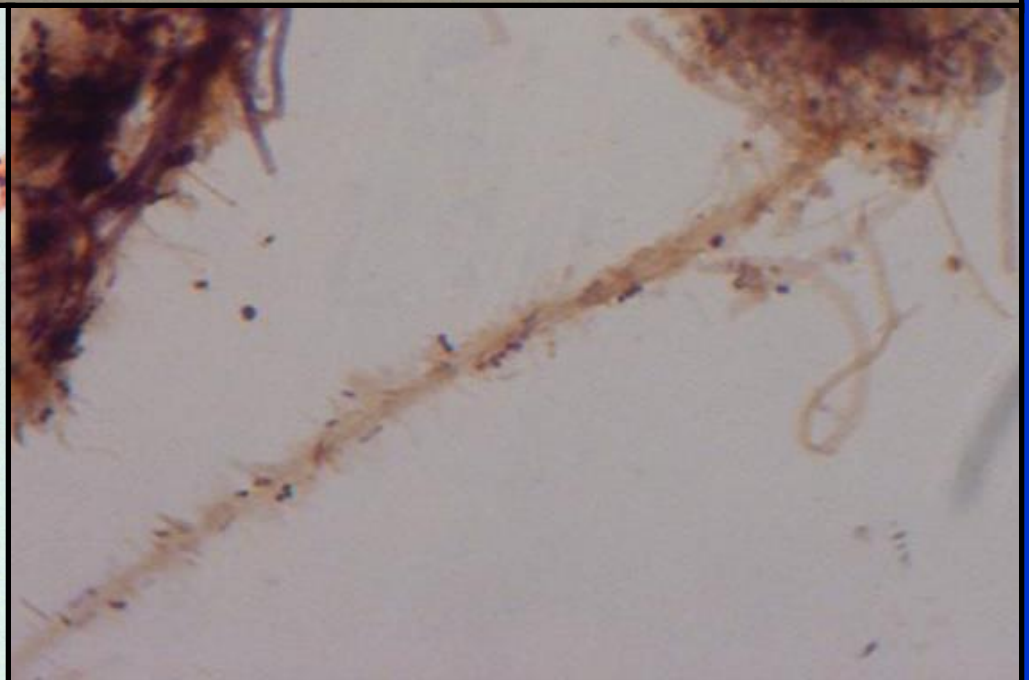
- **Filaments don't lie:**
  - **If you can identify the dominant filaments…**

…you can identify the growth conditions in the reactors

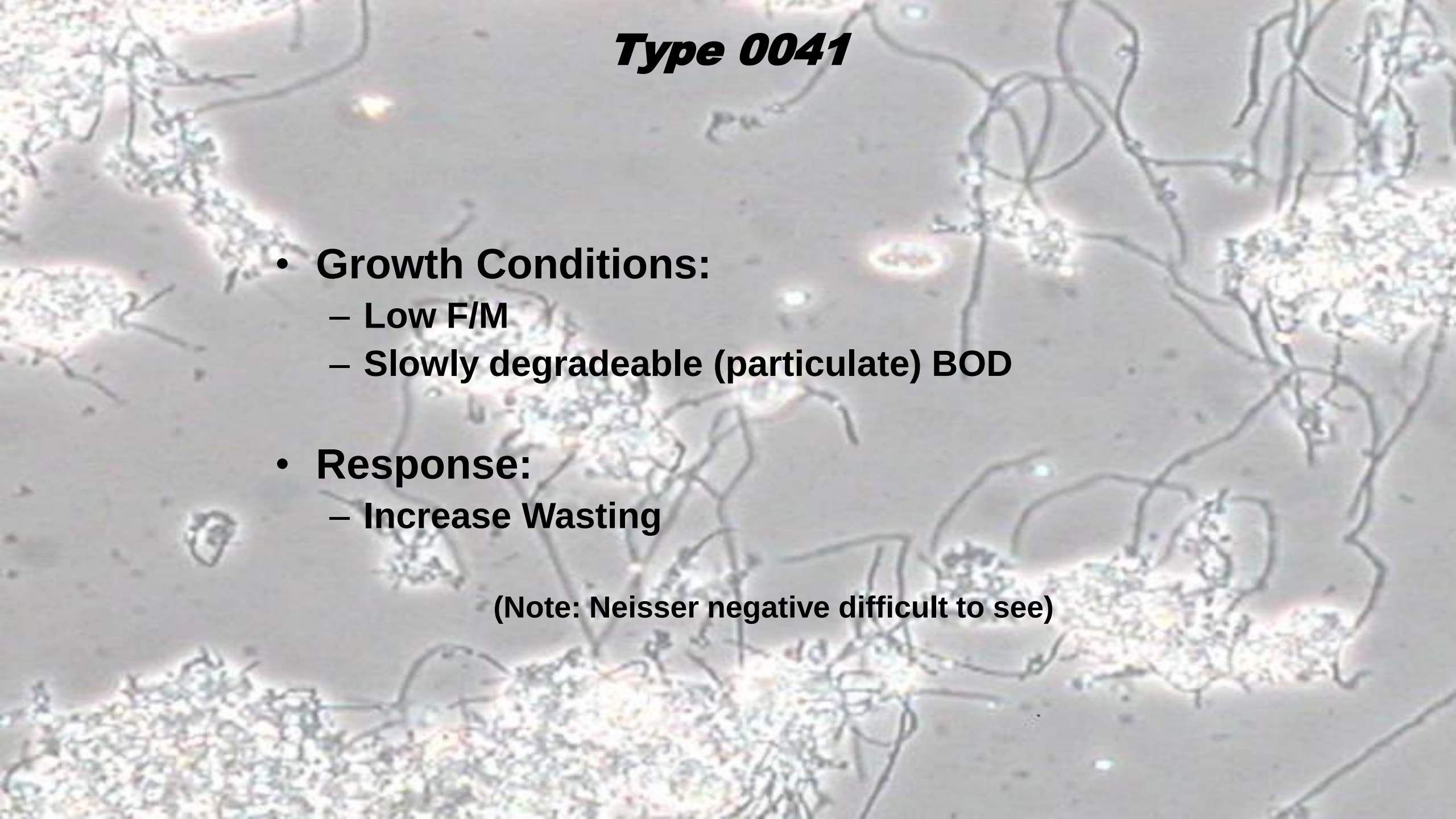
…and you can change the conditions in the reactors

…and eliminate the problem

Type 0041



Type 0041



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

(Note: Neisser negative difficult to see)


Type 1851

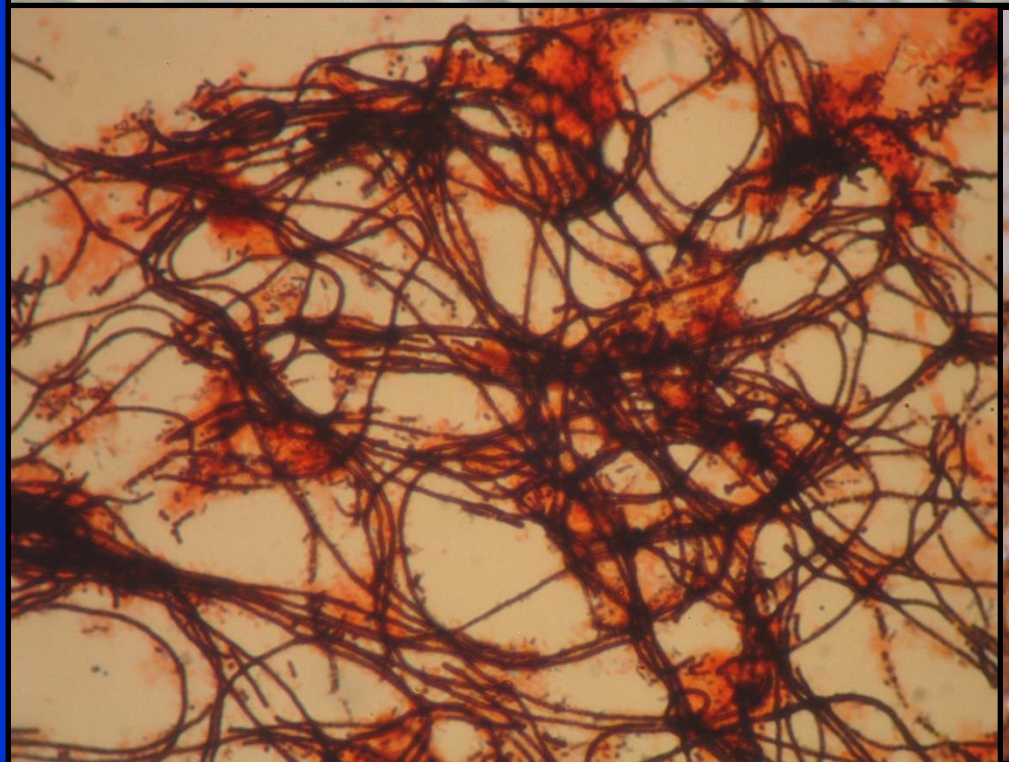
Type 1851

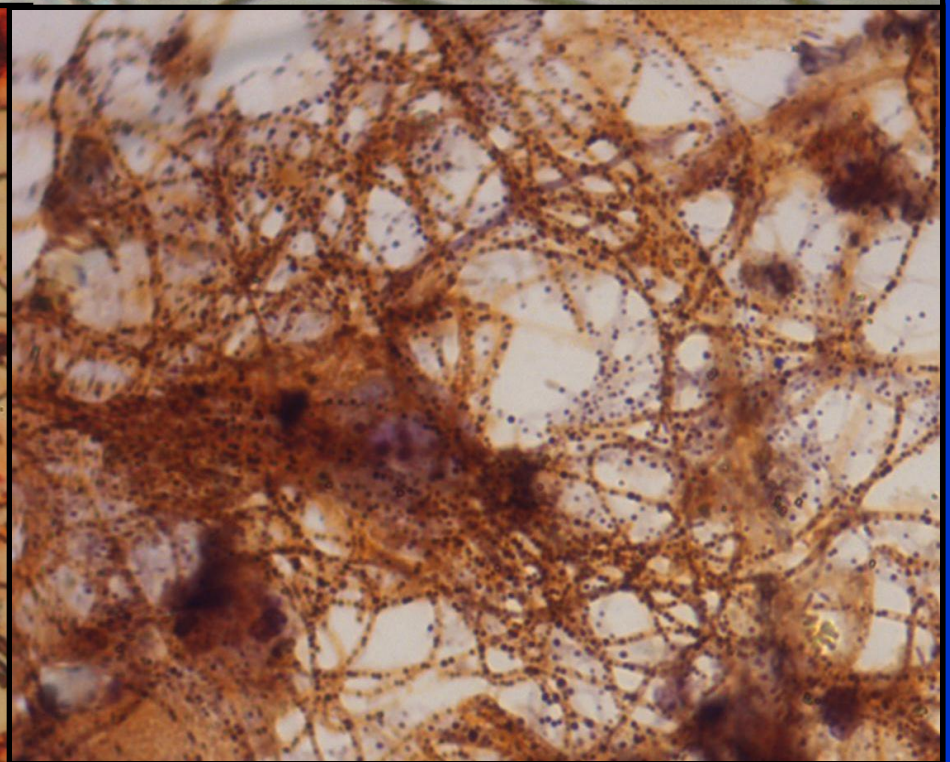
- **Growth Conditions:**
  - **Low F/M**
  - **Complete mix basin**

- **Response:**
  - **Increase Wasting**
  - **Plug flow / Selector**

(Note: Neisser negative difficult to see)


Microthrix Parvicella



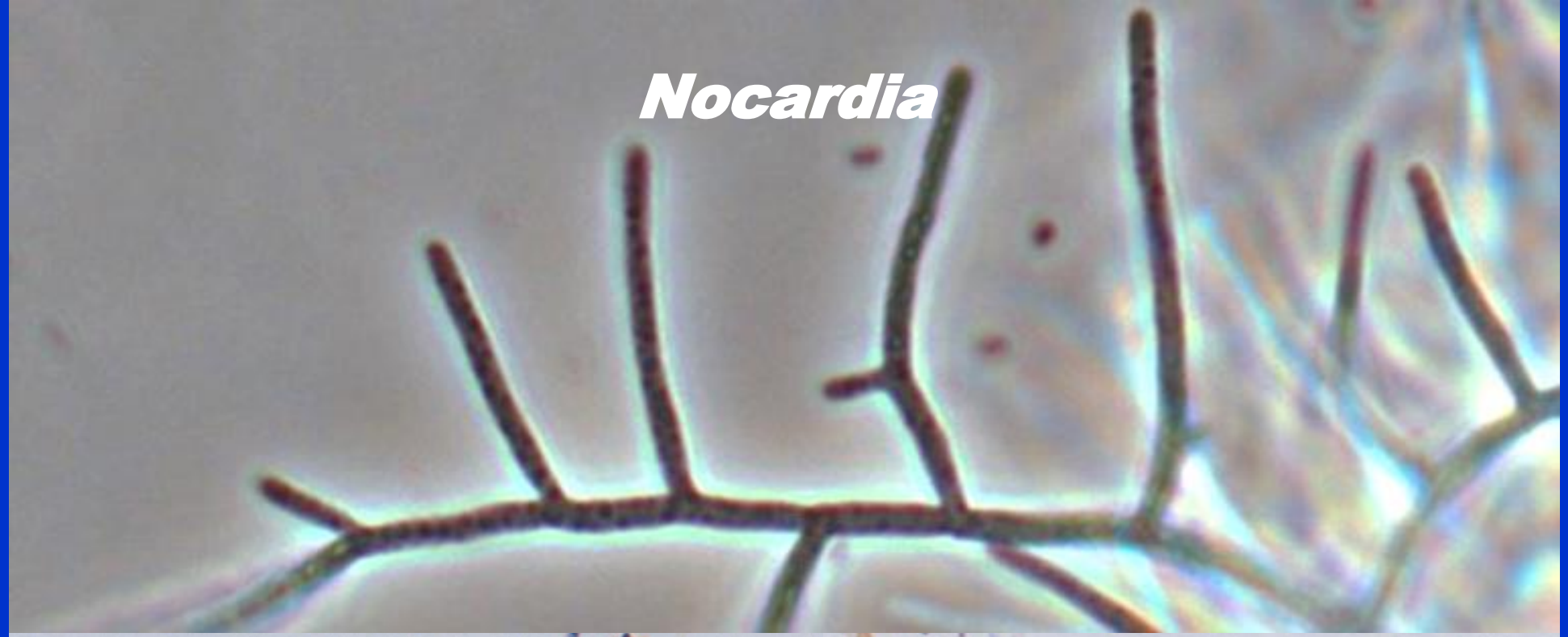


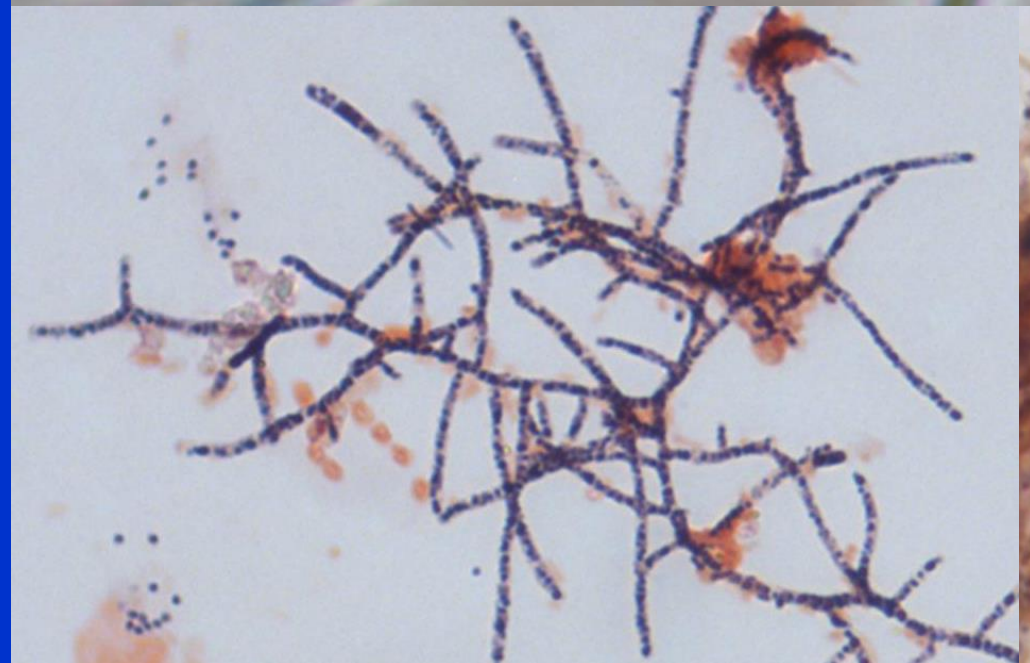
Microthrix Parvicella

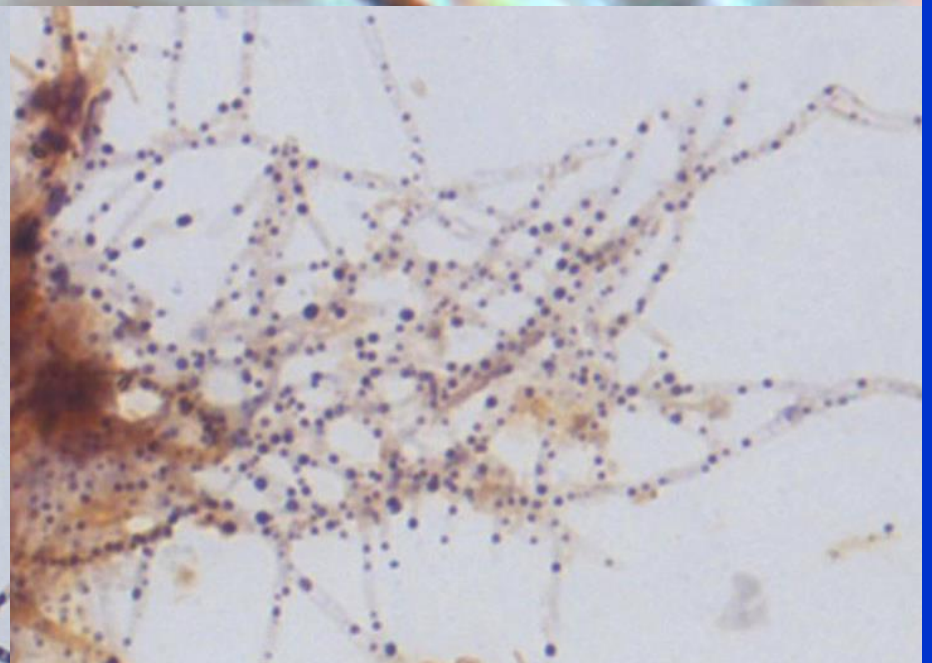
- **Growth Conditions:**
  - **Oil and Grease (lipids); High Carbon Chain Fatty Acids**
  - **Low F/M**
  - **Low DO**
  - **Cold water temperature**
- **Response:**
  - **Oil and Grease control (primary clarifier)**
  - **Foam trapping eliminated**
  - **Increase Wasting**
  - **Maintain adequate DO**

(Note: Neisser positive granules occur)

Nocardia







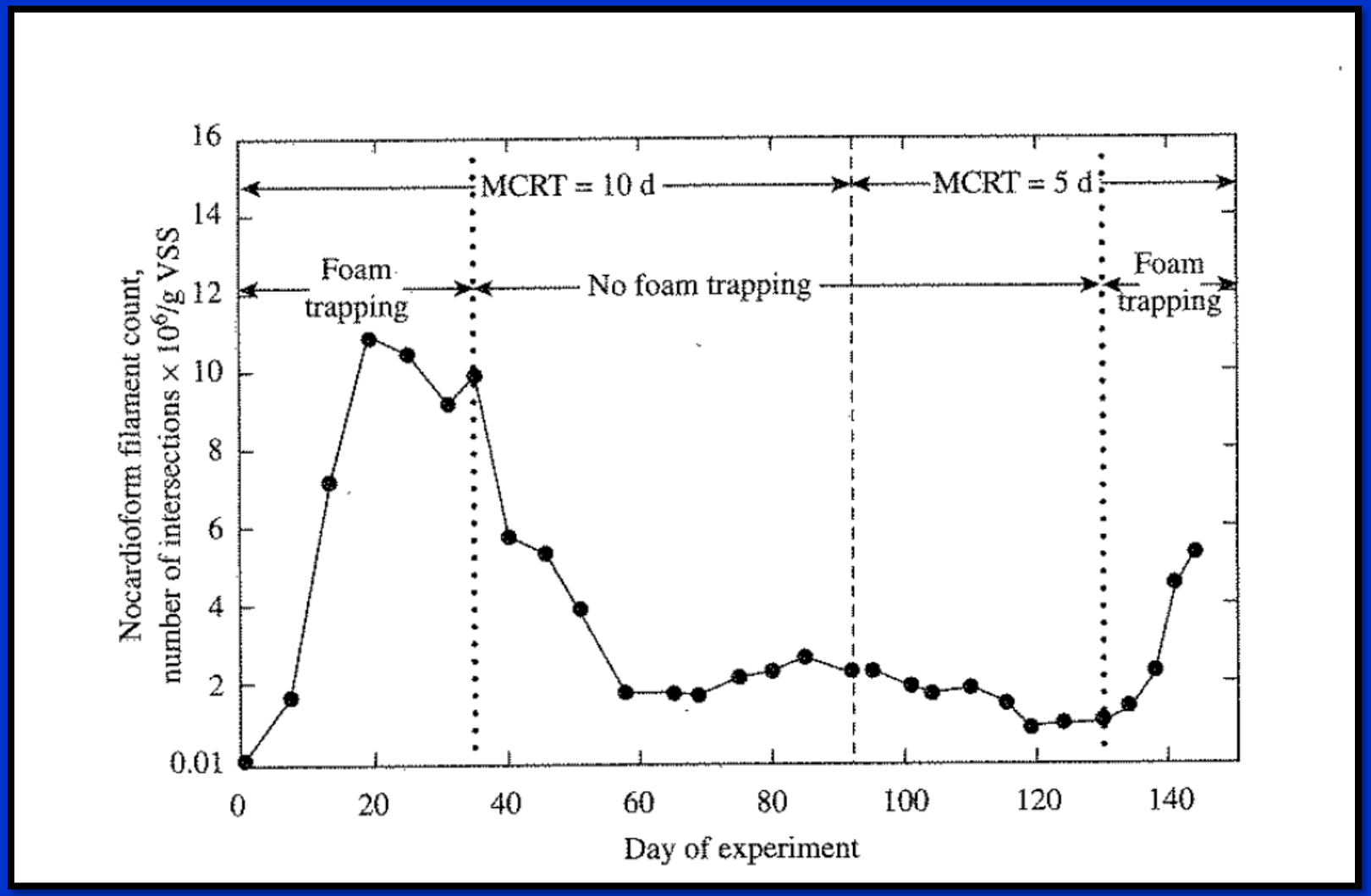
Nocardia

- **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**

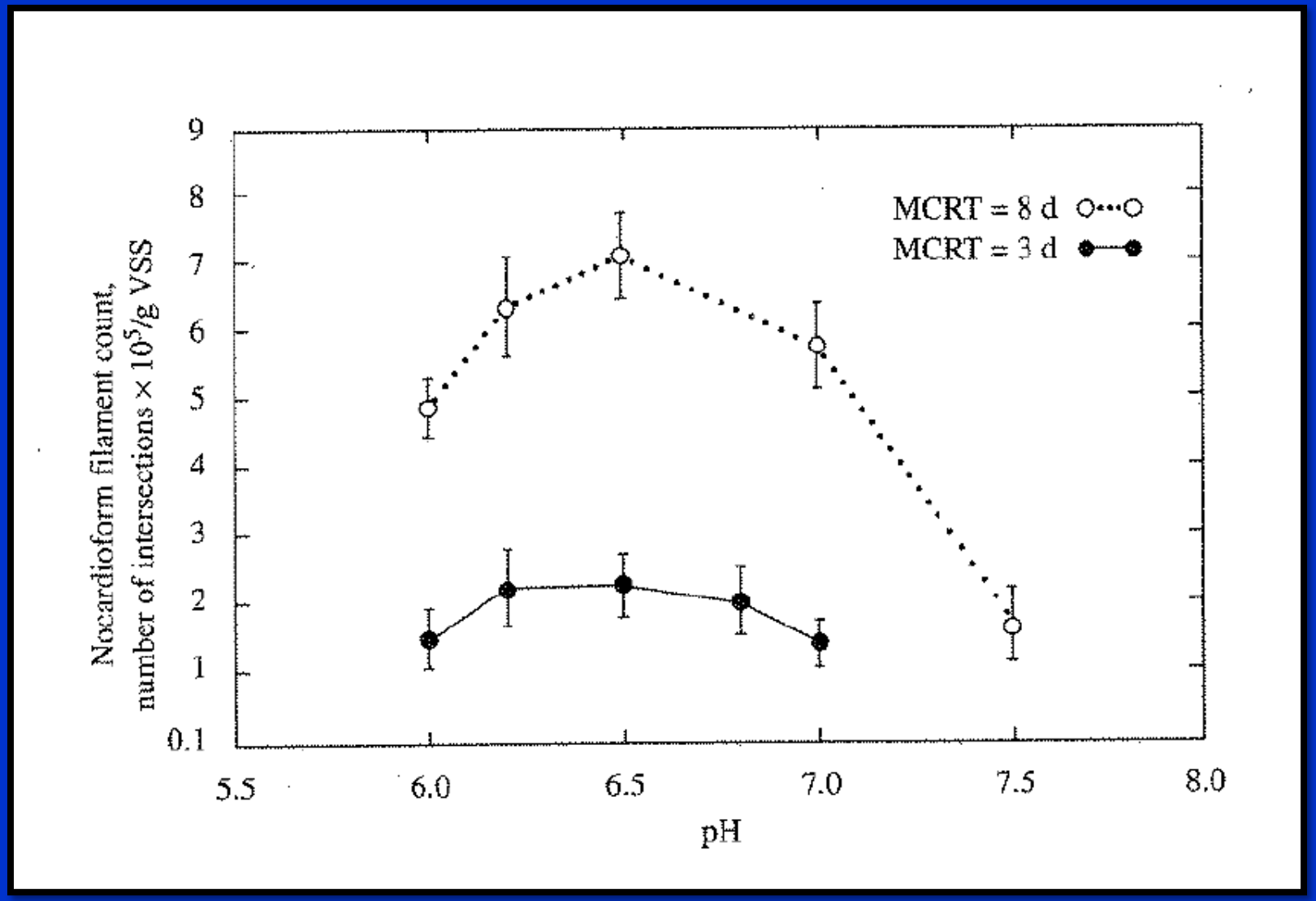
(Note: Neisser positive granules occur)

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.

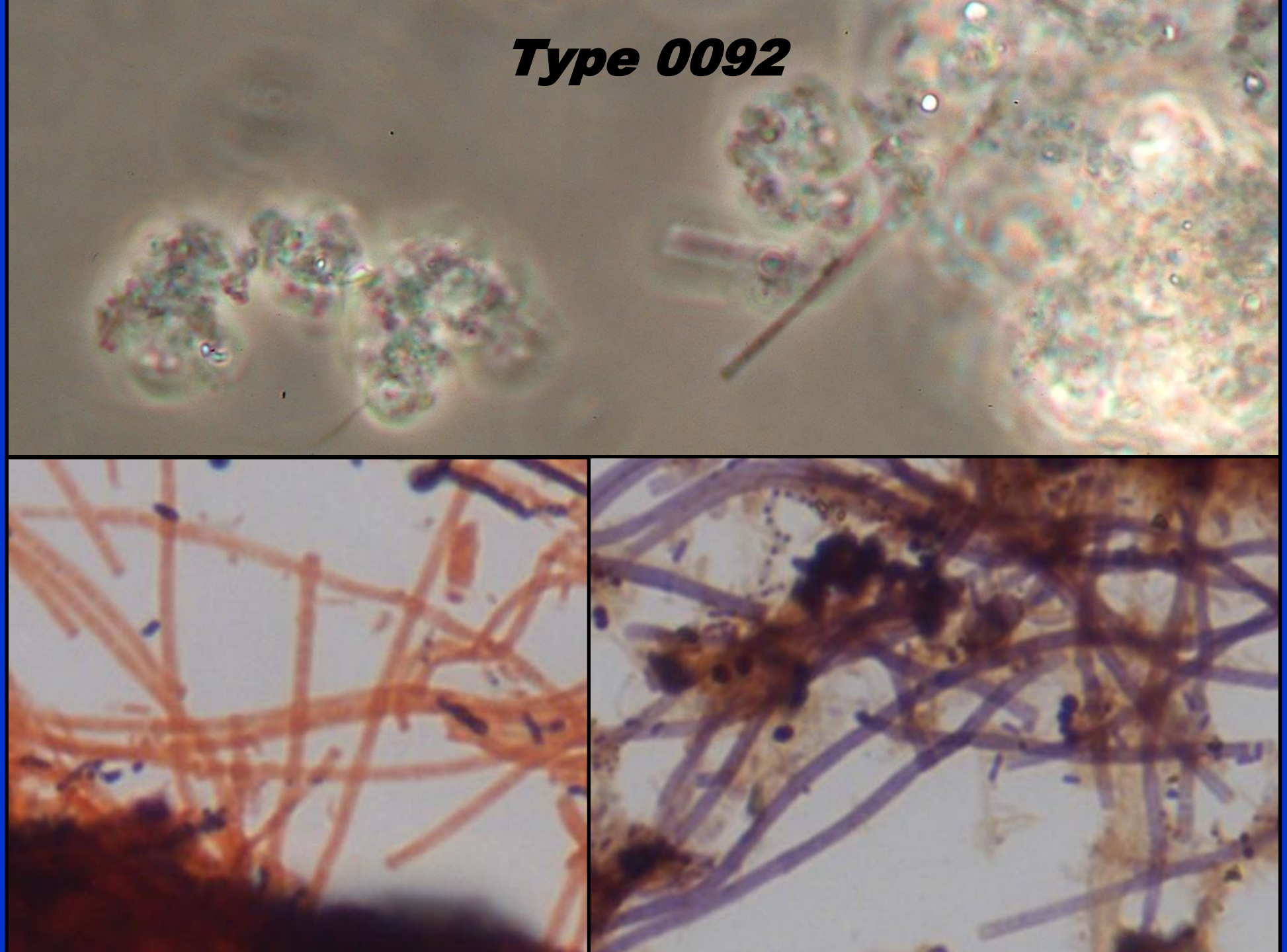
Maintain Short MCRT

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[a]	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	0.05
Type 1701	←						→		
S. natans	←						→		
H. hydrossis	←								→
Thiothrix spp.	←						→		
Type 021N				←					→
Nocardioforms		←							→
Type 0411		←					→		
N. limicola II			←						→
Type 1863	←	→							
Type 0041							←		→
Type 0675							←		→
M. parvicella							←		→
Type 0092								←	→
Type 1851							←		→
Type 0914							←	→	
Type 0803								←	→
Type 0581								←	→

[a] F/M as kg BOD_5/kg MLSS, d.

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


Type 0092

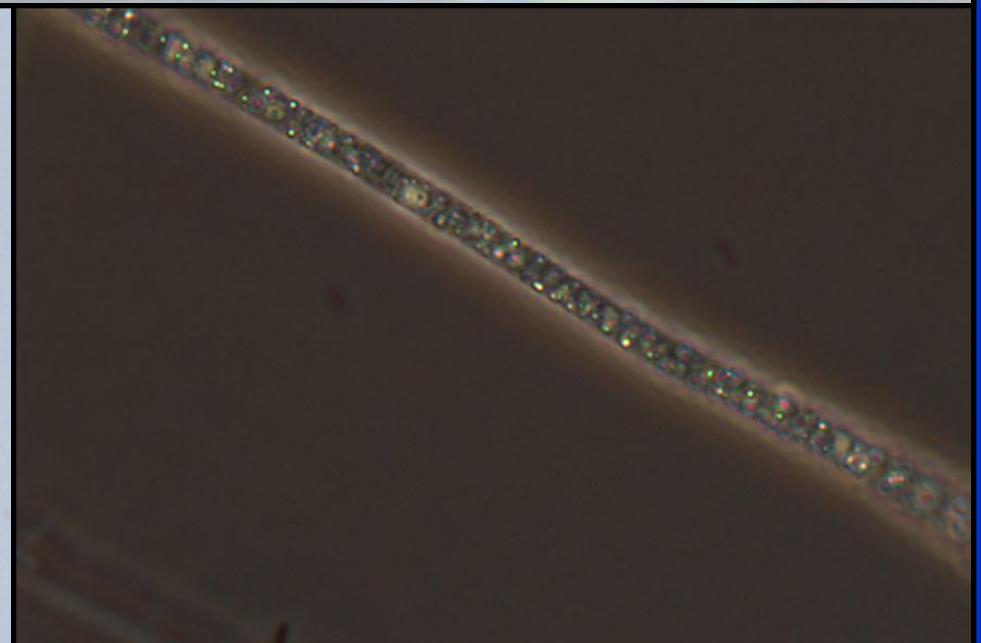
Type 0092

- **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

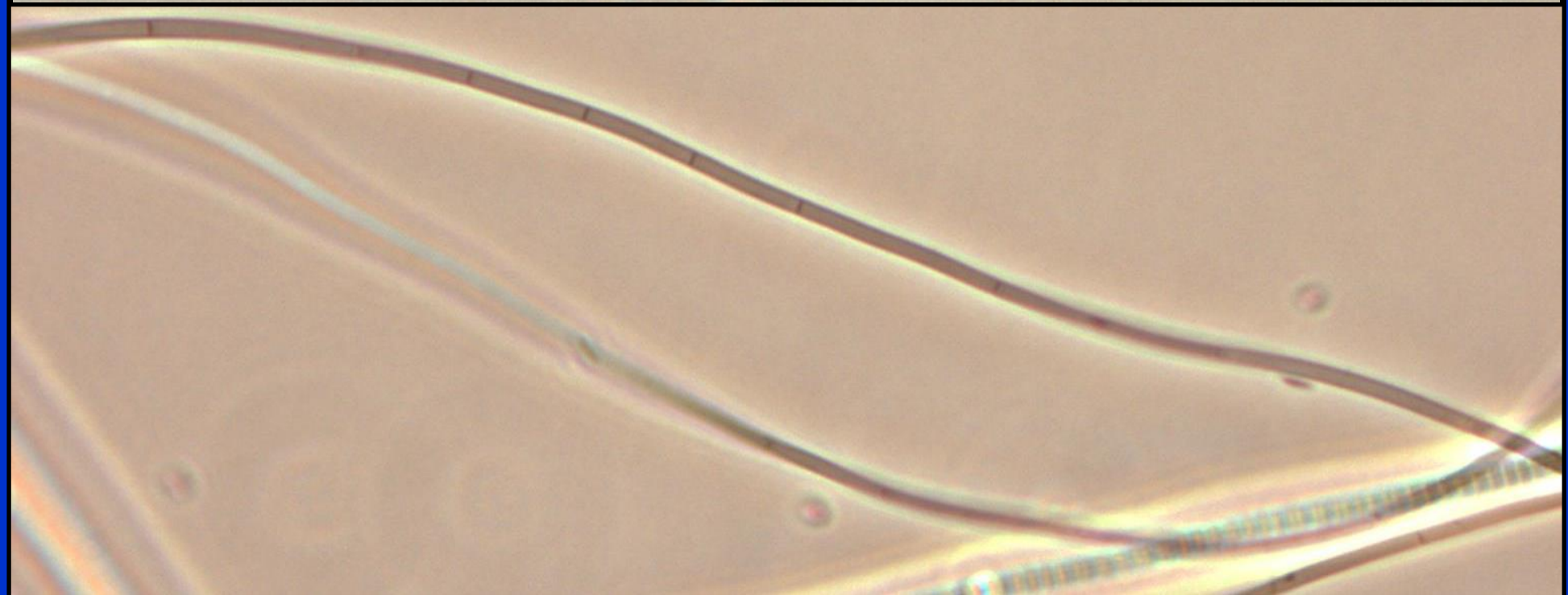
- **Growth Conditions:**
  - **Septicity, low molecular weight organic acids**
  - **Sulfides**
  - **Typically higher F/M environment**
- **Response:**
  - **Remove sources of septicity (long forcemains, excessive clarifier sludge blankets, digester decant)**
  - **Preaeration**

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**

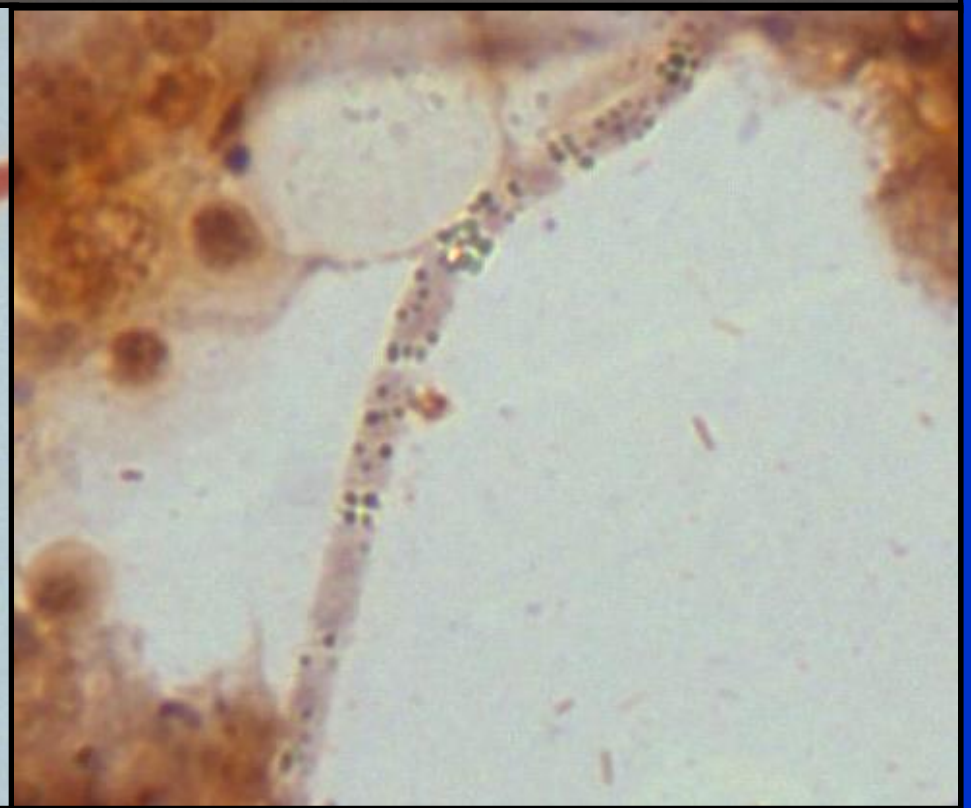
Type 0961



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

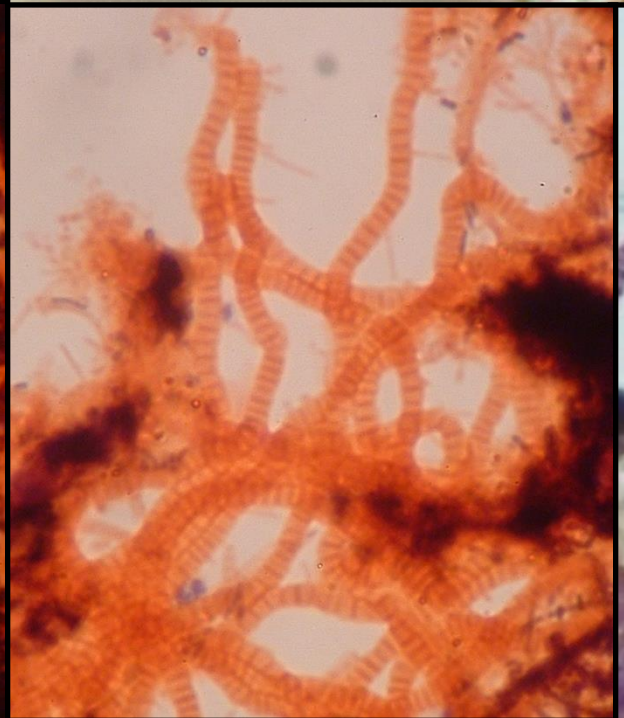


Beggiatoa

- **Growth Conditions:**
  - **Sulfides in wastestream**
  - **Lower Dissolved Oxygen**

- **Response:**
  - **Preaerate to remove hydrogen sulfides in wastestream**

Nostocoida Limicola

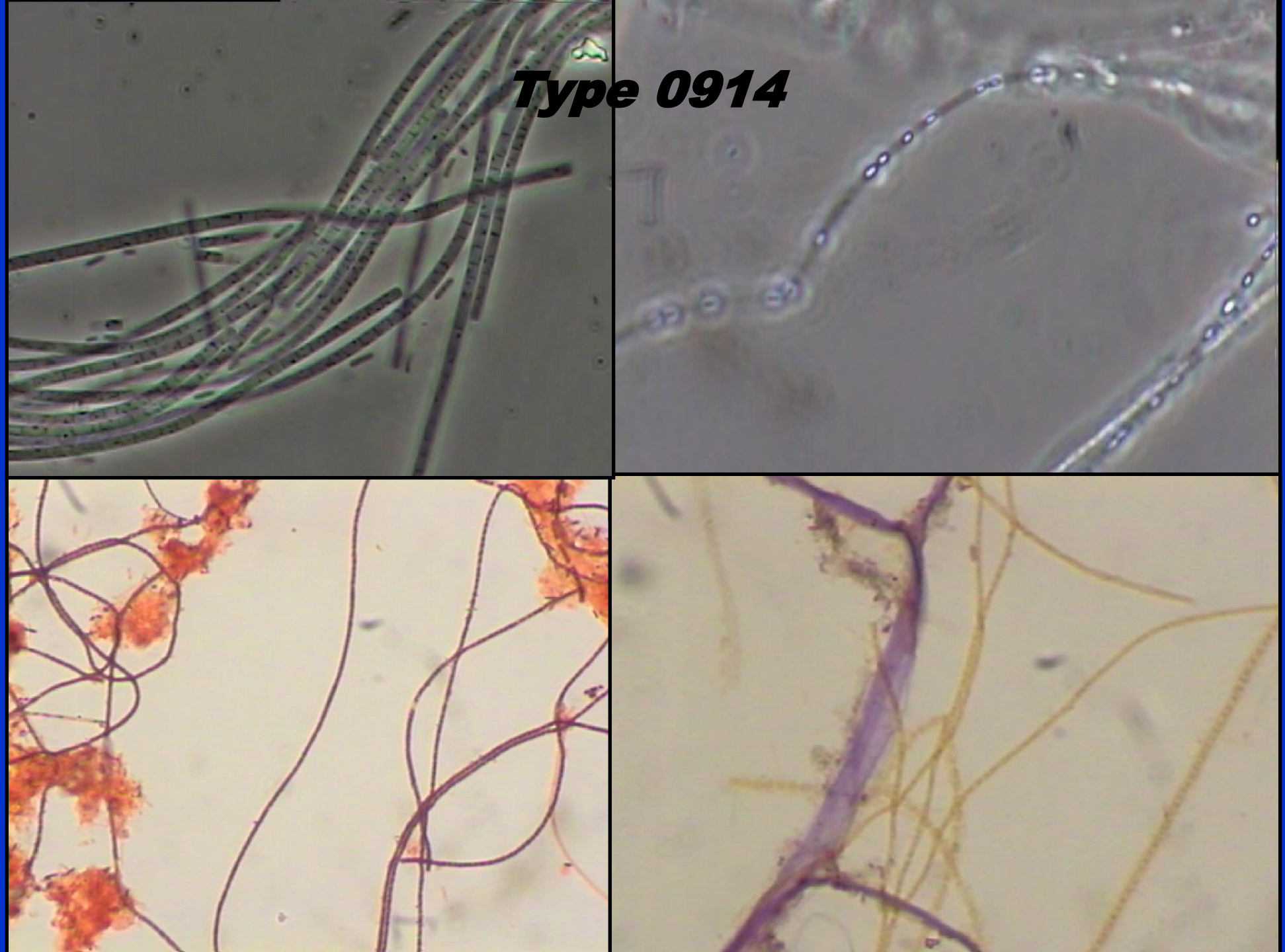


Nostocoida Limicola

- **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**

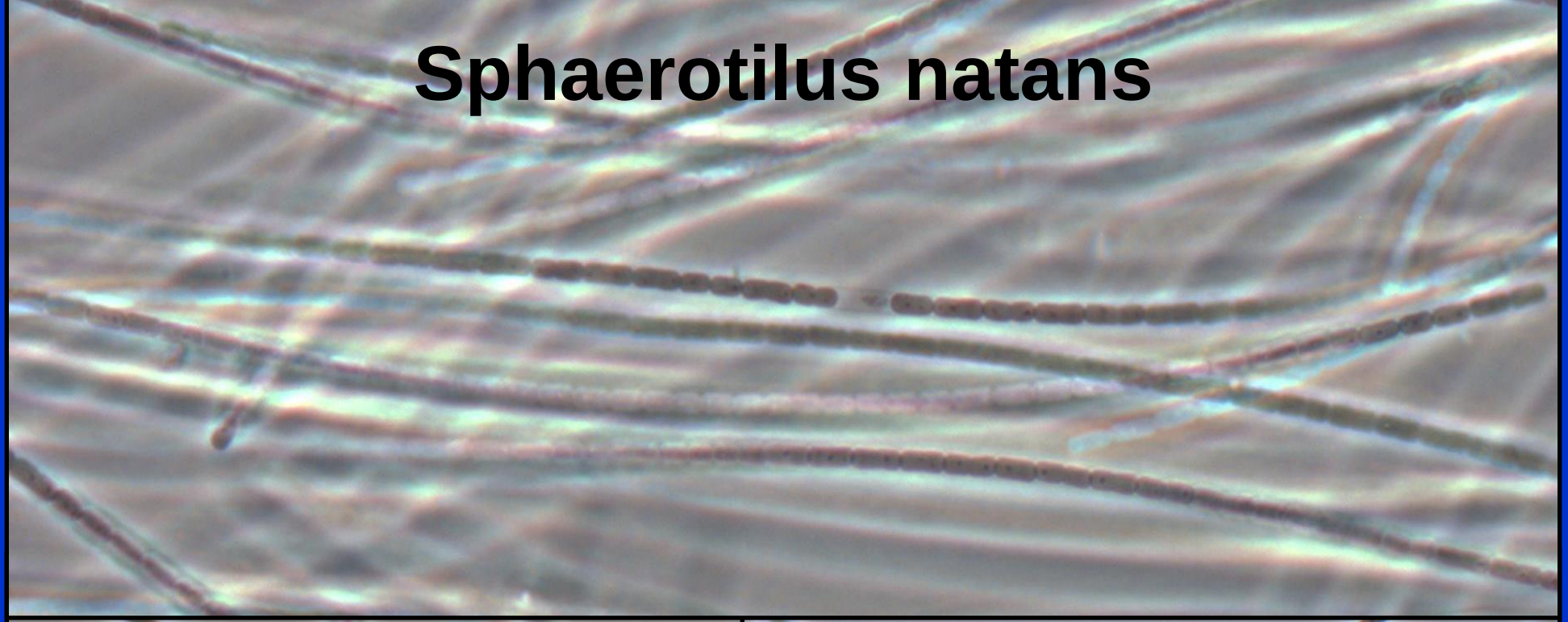
(Note: Gram and Neisser negative occur)

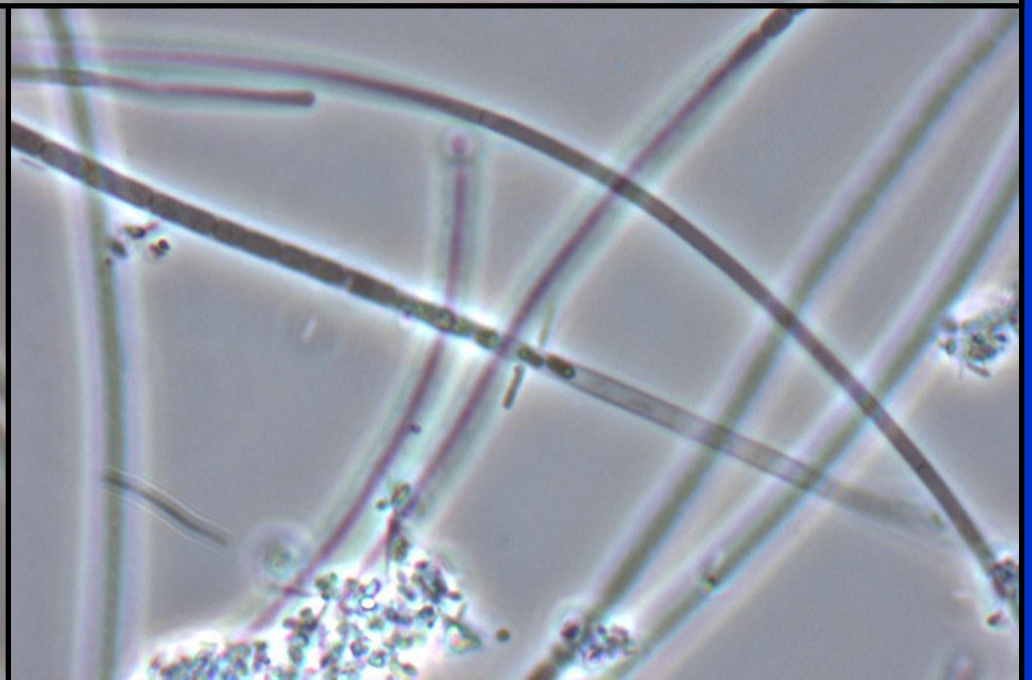

Type 0914

Type 0914

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

Sphaerotilus natans

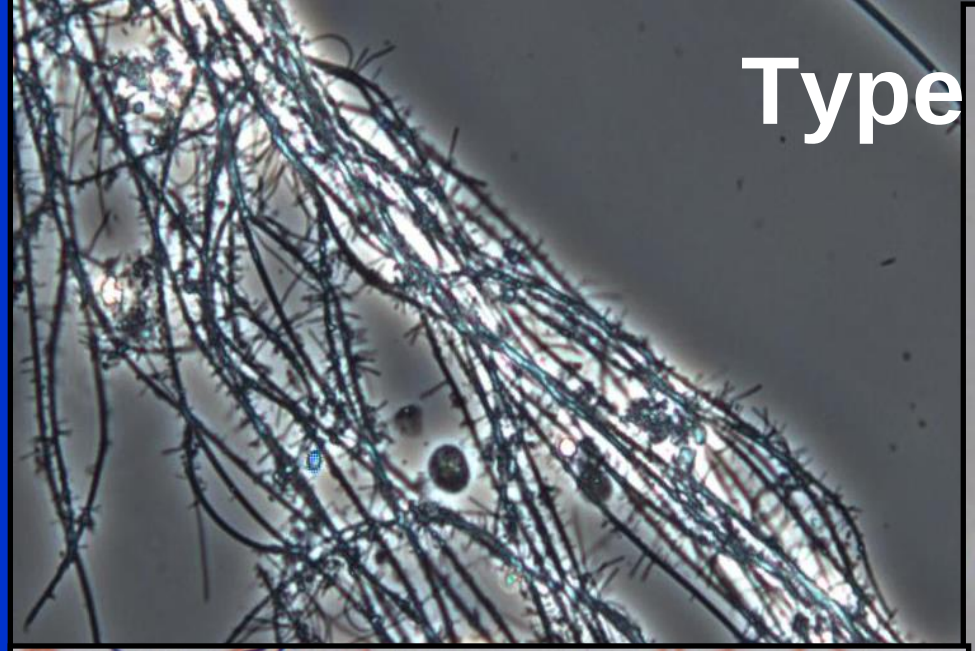


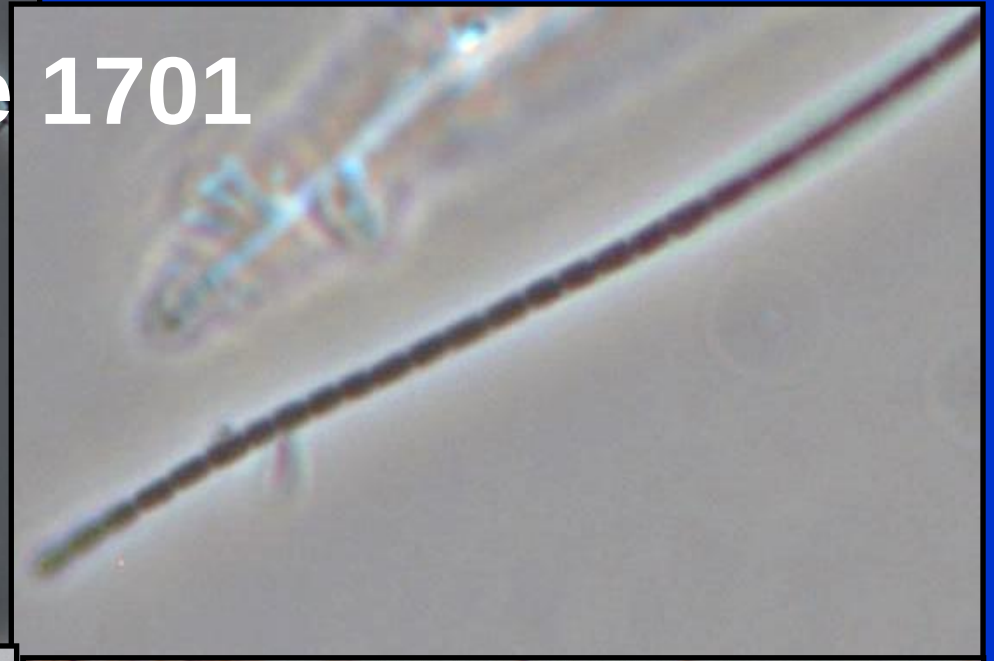


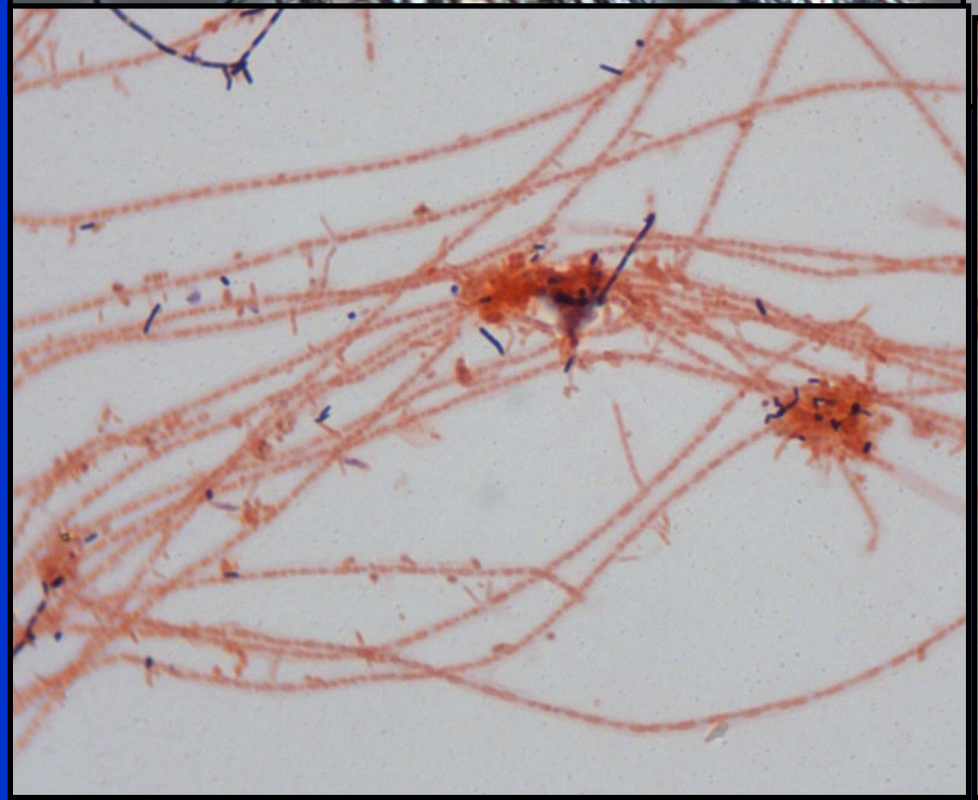
Sphaerotilus natans

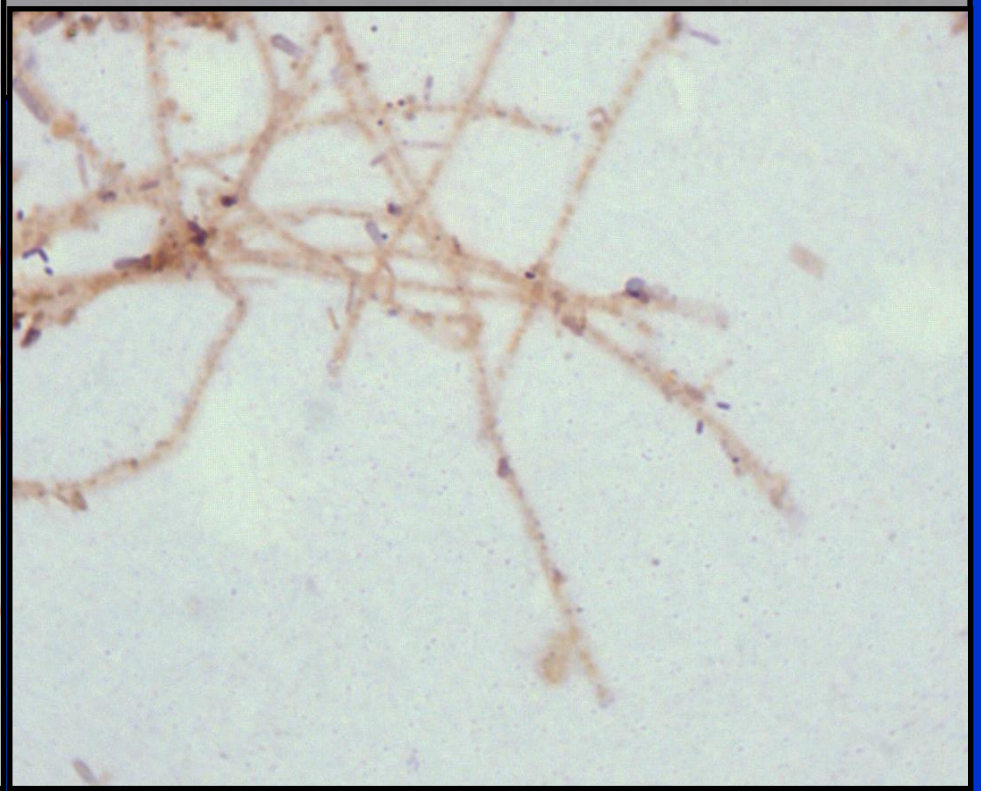
- **Growth Conditions:**
  - **Low Dissolved Oxygen**
    - **Low DO for the applied load**
    - **Low DO in the interior of the floc**
- **Response:**
  - **Increase bulk Dissolved Oxygen in Aeration**

Type 1701







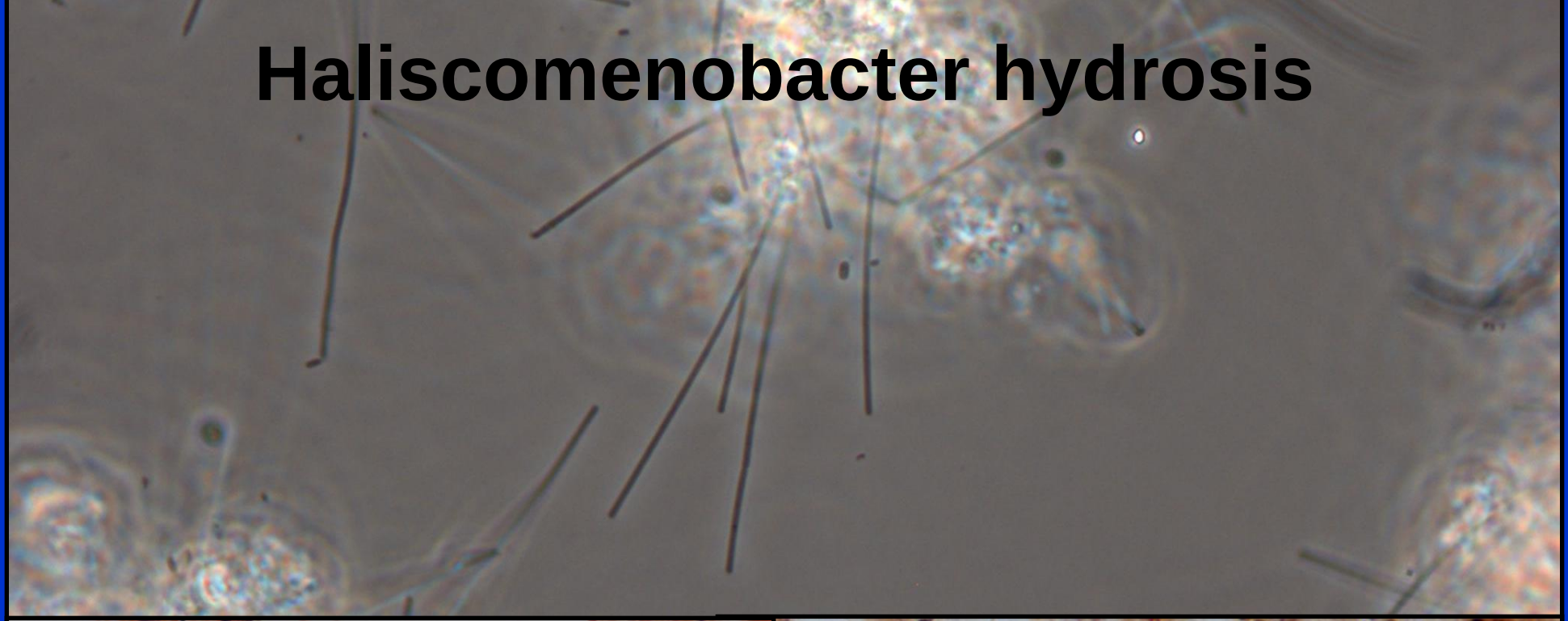


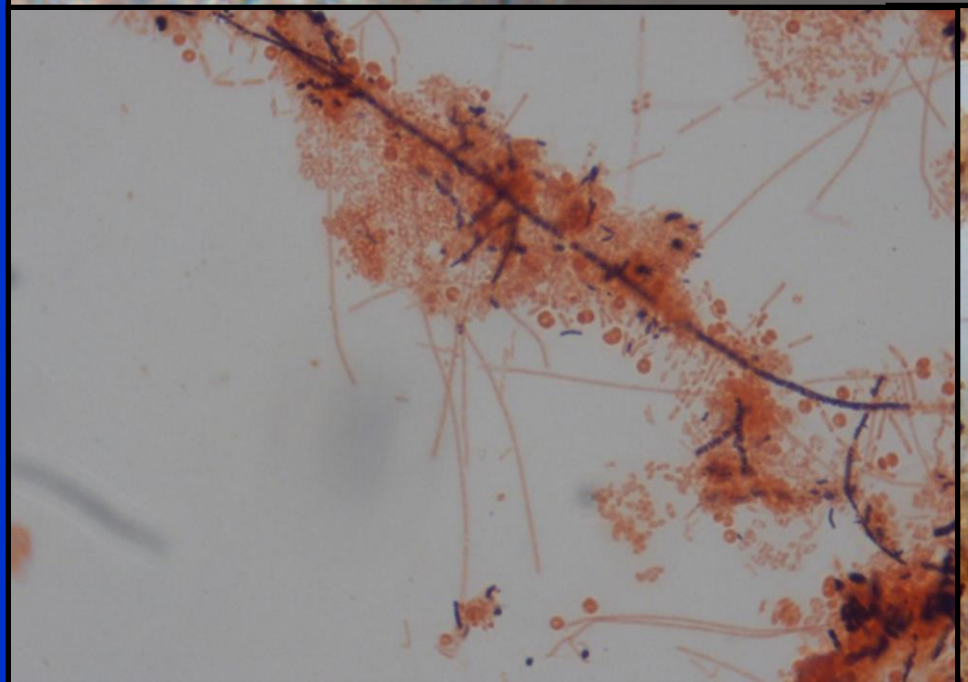
Type 1701

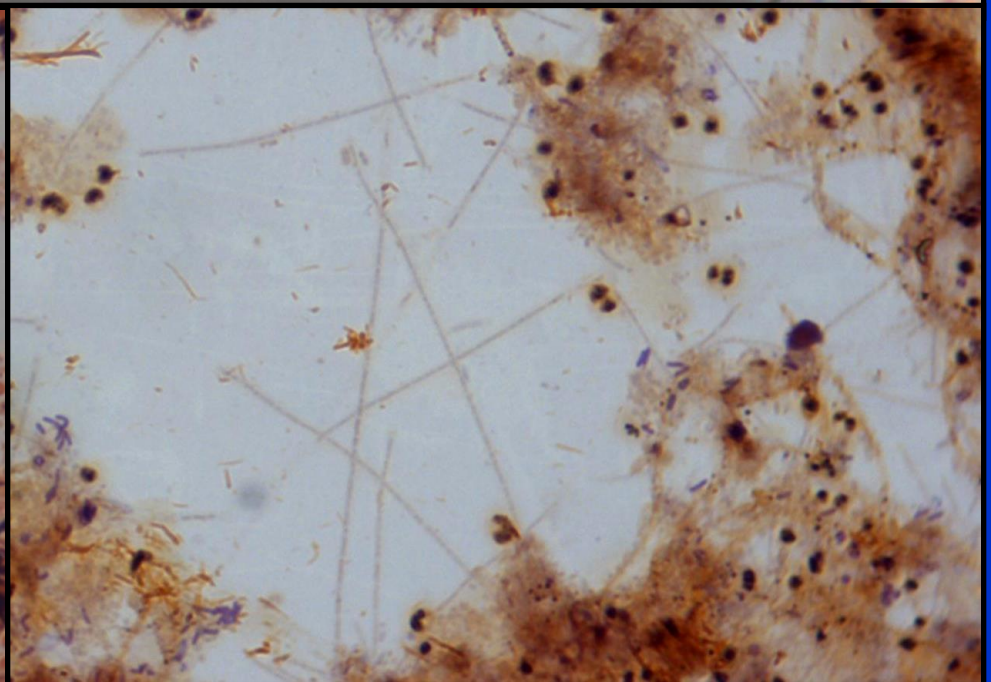
- **Growth Conditions:**
  - **Low Dissolved Oxygen in Aeration Tank**
  - **Wide range of F/M**

- **Response:**
  - **Increase Dissolved Oxygen in Aeration Tank**

Haliscomenobacter hydrosis







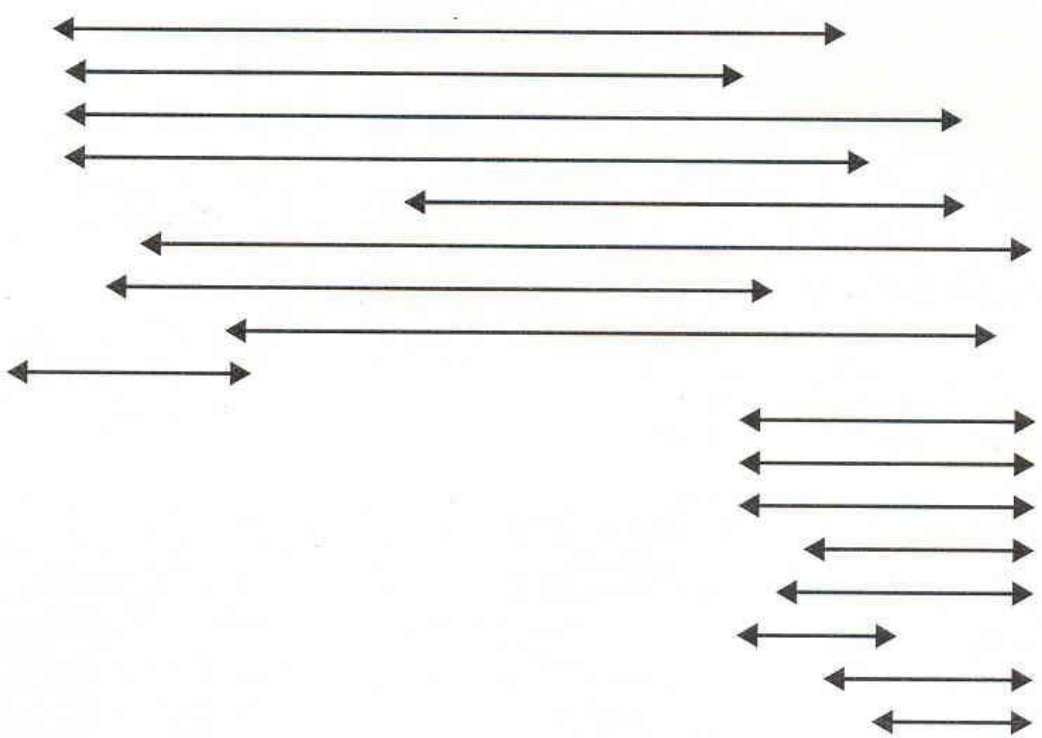
Haliscomenobacter hydrosis

- **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**

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[a]	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	0.05



[a] F/M as kg BOD_5 /kg MLSS, d.

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

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

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

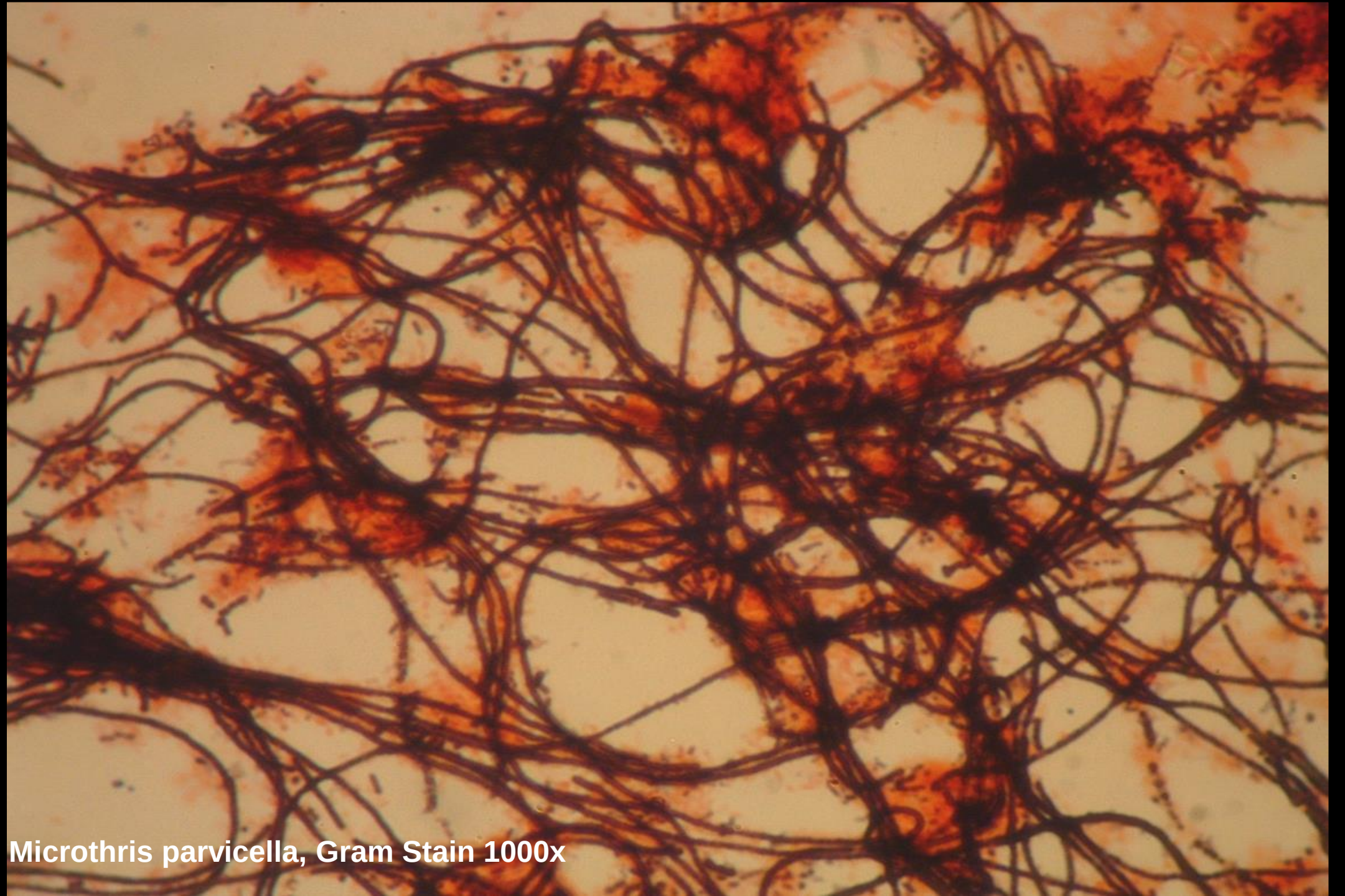
- Microscope Slides
- Clothes pin
- Wash bottle
- Watch with a second hand
- Paper towel
- Sink (preferably a black lab sink) or roasting pan
- Bon Ami Scouring Powder (optional)

Tools for Staining

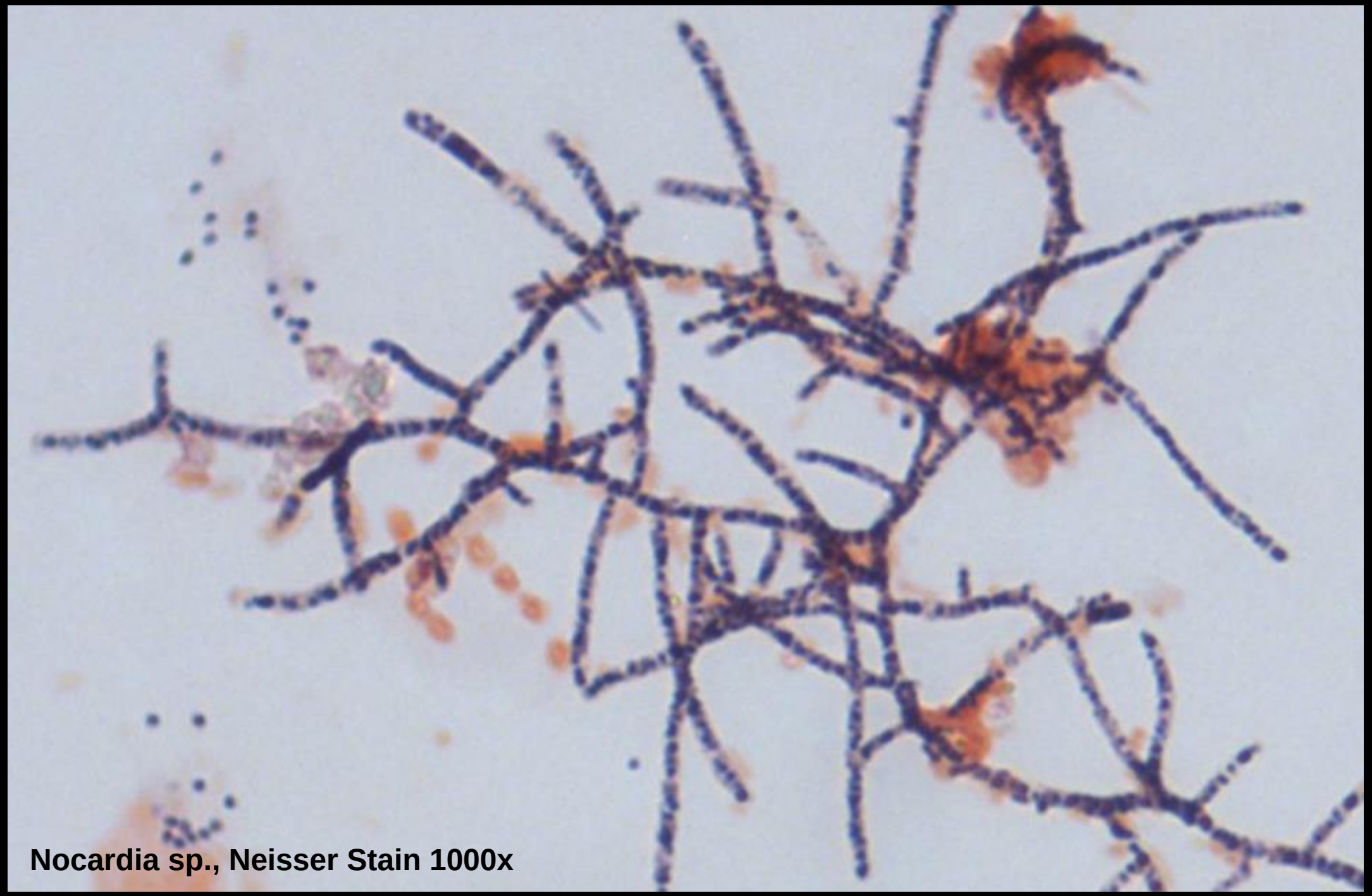
- **Gram Stain Kit**
  - **Gram Crystal Violet Solution**
  - **Gram Iodine Solution**
  - **Gram Decolorizing Solution**
  - **Gram Safranin Solution**

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)**



Microthris parvicella, Gram Stain 1000x



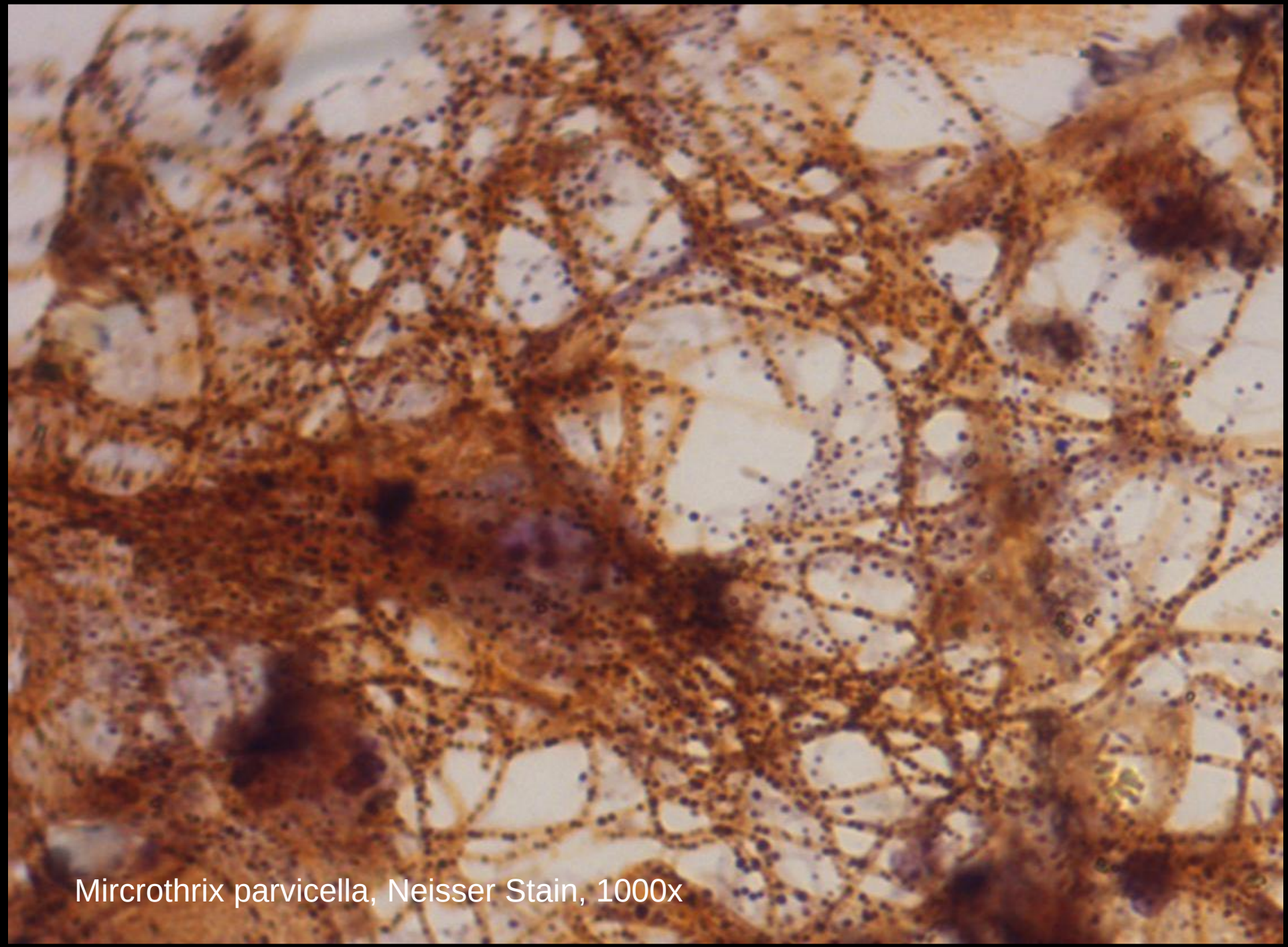
Nocardia sp., Neisser Stain 1000x

Tools for Staining

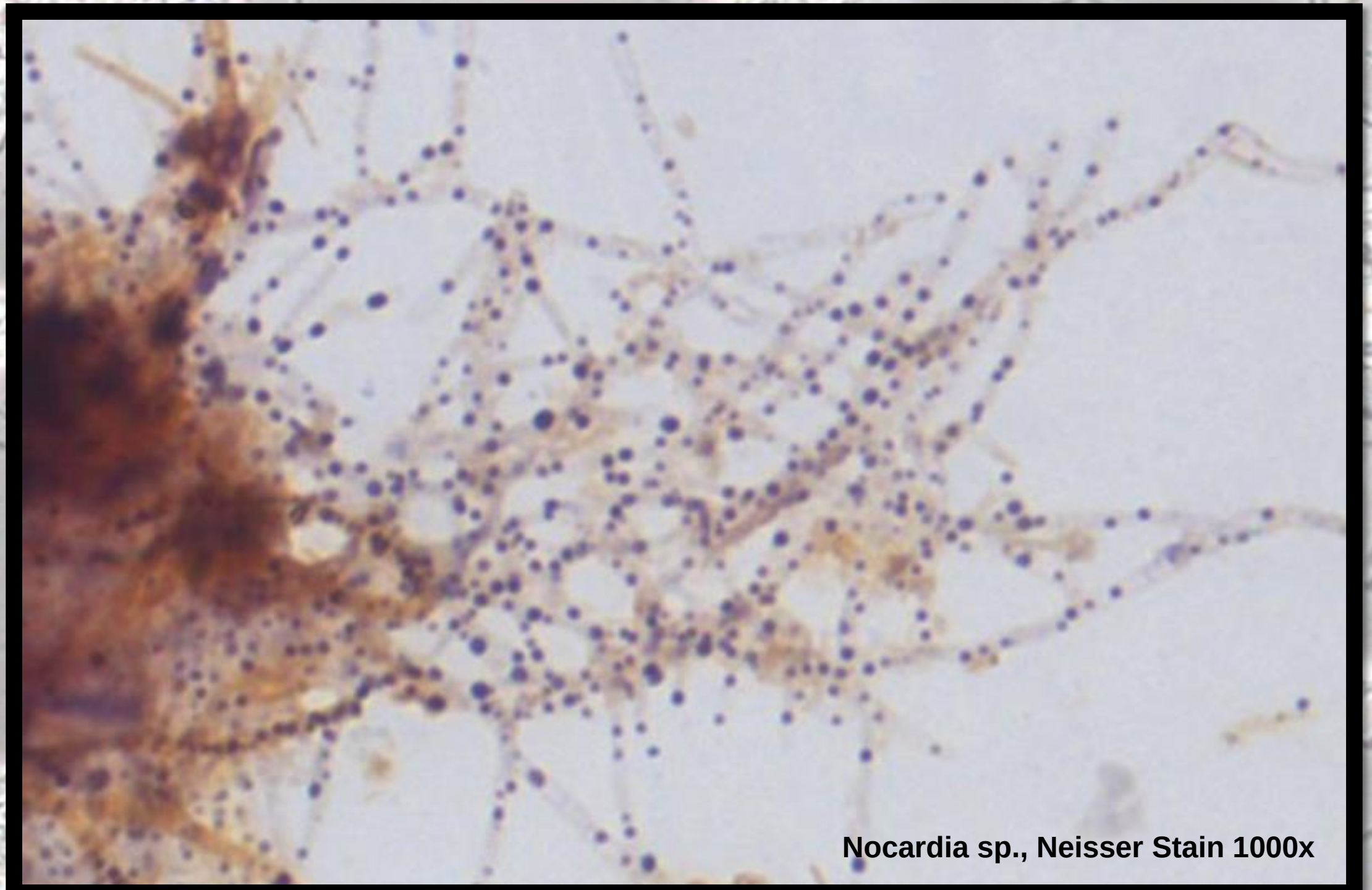
- Neisser Stain Kit
  - Neisser Methyl Blue Solution A
  - Neisser Crystal Violet Solution B
  - Neisser Bismark Brown Solution
  - Transfer pipet
  - Container for mixing Solutions A and B
  - And the Clothes Pin, of course

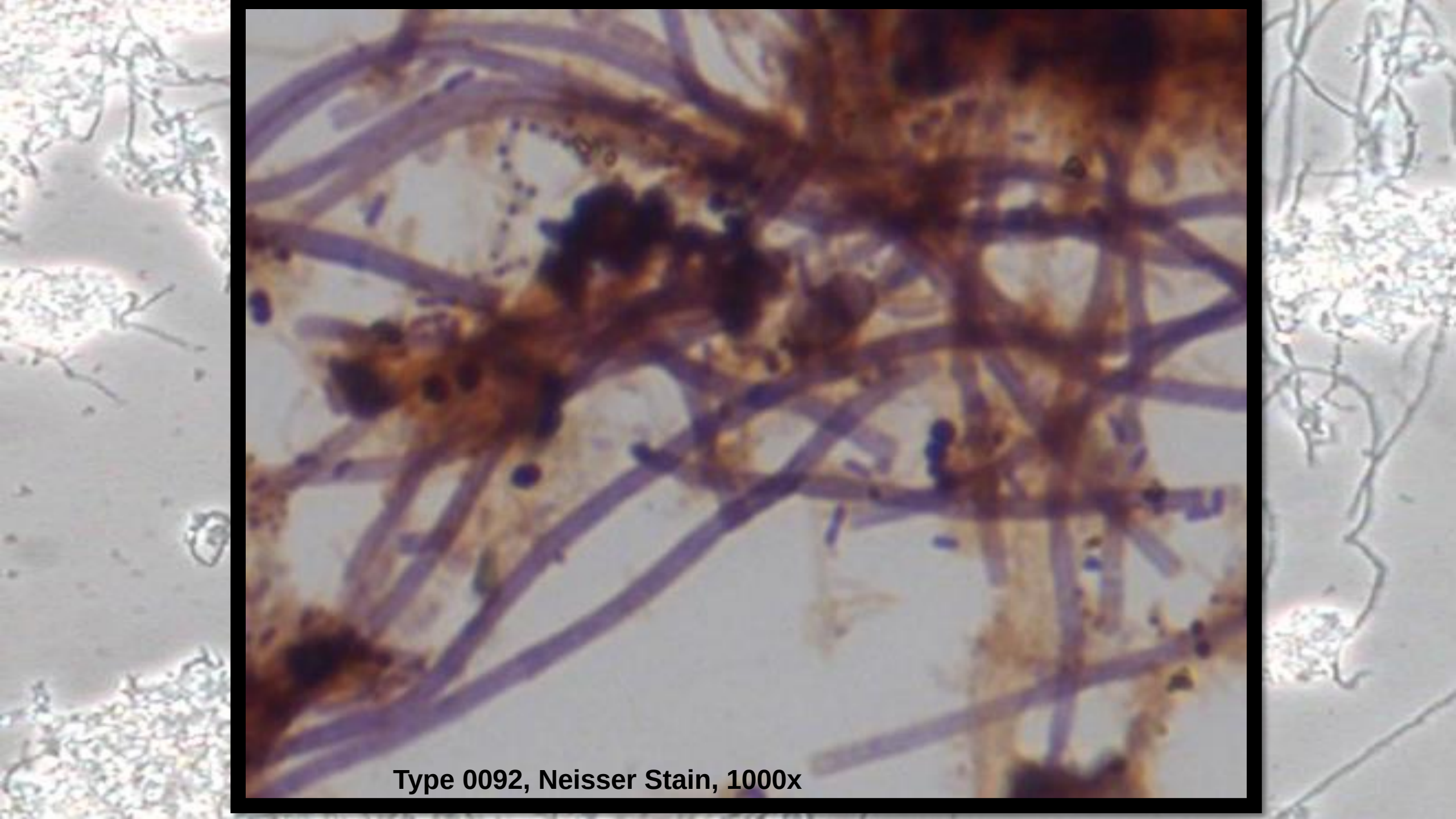
Neisser Staining Procedure

1. **Methyl Blue / Crystal Violet Solution**
   - Mix 2 parts Methly 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)**



Mircrothrix parvicella, Neisser Stain, 1000x


Nocardia sp., Neisser Stain 1000x



Type 0092, Neisser Stain, 1000x

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

Questions?

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