Basic Microscopy for Wastewater Operators

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Basic Microscopy for Wastewater Operators

7 Contact Hours
9 CC10 Hours

This program has been developed to help wastewater operators obtain improved process control through microscopic examination of mixed liquors and other waste streams. Starting with the basics, participants will be introduced to microscope features and benefits, the microscope selection process, and cost factors. The course includes an overview of sampling, slide preparation, maintenance, staining techniques, and sample examination. The course will also cover organism identification and the effects of the presence, absence, mobility, and organism type on wastewater process control.

1. Identify the different types of microscopes available for examination of mixed liquors;
2. Prepare a slide for examination;
3. Identify presence or absence of organisms in the prepared slide; and
4. Discuss techniques used for sample examination

Agenda

8:00 am –	Introduction/Pre-test
9:00 am -	Basic microscope features
10:00 am -	Optional features and value
10:30 am -	Choosing the right microscope to view
11:00 am -	Organisms
12:00 pm -	Lunch
1:00 pm -	Microscope basics-cover slide prep to
2:00 pm -	proper magnification tools
2:30 pm	Organism identification
3:30 pm -	Process Control Application
4:00 pm -	Evaluations/Post-Test

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Basic Microscopy for Wastewater Operators

By Mike Harrington, CET

Your Instructor

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Our Agenda

- Basic microscope features
- Optional features and value
- Choosing the right microscope for both water and wastewater applications
- Microscope preparations, slides, Petri Dish, etc

Types of Microscopes

Stereo Microscope

Compound Microscope

Potable Water Microscopy

Water Applications

- Uses 55 mm Petri dish
- Counts colonies of microorganisms
- Direct counting: microscopy; cytometry
- Culturing so that the biomass becomes visible
- Reporter assays where metabolic components are measured: colorimetry

Counting Mechanics

- Water is filtered on a filter which has holes smaller than bacteria
- The filter is then placed on a dish of agar and bacteria grow on the top of the filter.
- Count the bacteria and calculate the cell concentration (titer)
- One big advantage of this method is that huge volumes of water can be filtered to show a few bacteria per liter.

Interpretation

- Suppose 125 colonies grow in the petri plate. Multiply the dilution factors by the count to obtain the titer of the bacteria sample: 100 x 100 x 20 x 125

Stereo Microscope Uses

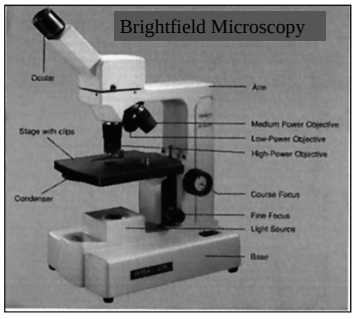
- Low power applications
- Bacterial colony counting etc.
- Binocular
- Macro-specimen viewing
- QA/QC
- Usually 5X to 35X in magnification
- Three-dimensional images
- Good width & depth of field

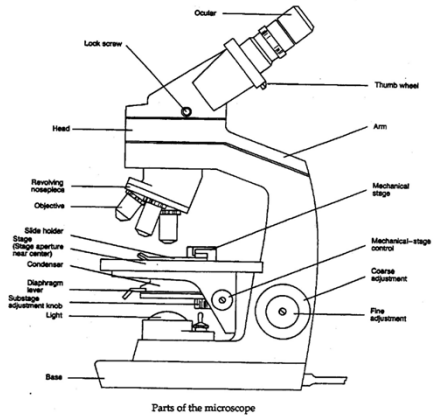
Wastewater Microscopy

Compound Microscope

- Magnifies very small objects
- Thin specimens to transmit light
- Specimens mounted on slides
- High quality specimen selection & preparation important
- Correct selection of magnification and lighting crucial
- Available options: Phase Contrast, adjustable condensers, Dark-field

Compound Microscope





Parts of the microscope

Bright-field Microscopes

- Optical range from 40X to up to 1000X
- Ideal for larger populations
- Large filamentous counting
- Large rod-shaped bacteria populations
- May miss smallest protozoa's & bacteria

Bright-field Microscopes

Binocular Brightfield with mechanical stage

Use of Bright-field Microscopes

- Well suited for pigmented specimen with contrast
- Useless on colorless bacteria, tissue, single organisms
- Good for stained bacteria, grouped colonies
- Living organisms
- magnifications 40X, 100X, 400X, 1000X

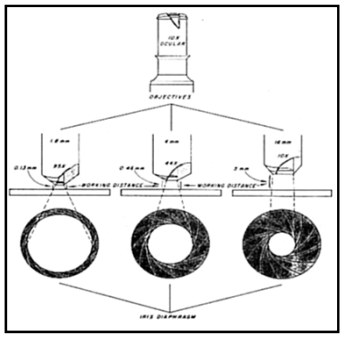
Special Bright-field Features

- Spring-loaded 40X and 100X objectives, protects cover & slide.
- Parfocality- switch power w/ limited re-focusing
- Stage mounting and locking for slide
- Upgradeable

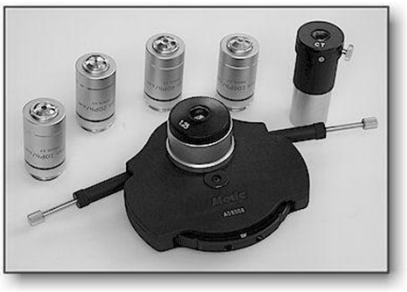
MAGNIFICATION

- Two-lens system
  - Ocular (Closest the eye)
  - Objective(Closest the specimen)
- Total Magnification =
  - Ocular rating X Objective Rating

Magnification



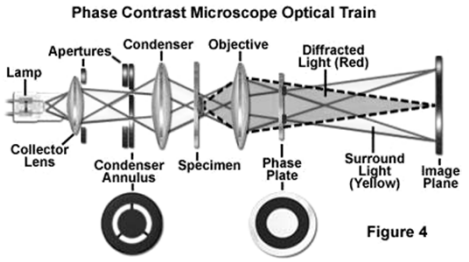
Phase Contrast Microscopy



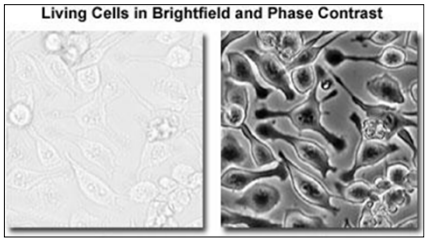
Phase Contrast Microscopes

- Light bends to greater angles, away from center of lens where intensity is needed
- Results in less visual detail in living cells
- Preferred in 400X to 1000X magnification to see cell detail.
- Ideal for smallest Protozoa
- Rod-shaped bacteria
- Certain algae

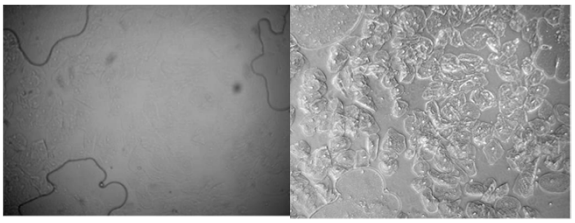
Phase Contrast Imaging



Brightfield / Phase Contrast



Brightfield / Phase Contrast



Human cheek cells shown

Phase Contrast with Staining

- Improves viewing of organism cell structure
- Aids in Identification
- Staining usually kills the organism
- Phase microscopy does not
- More organism information

Darkfield Microscopy

- Opaque disk added under condenser
- Only light that is scattered reaches the eye
- Use in low magnification-100X
- Ideal initial investigation of Mixed Liquor
- Determine motile/nonmotile bacteria
- Algae
- Protozoan scans

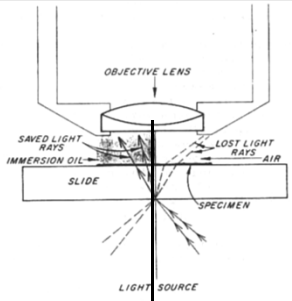
Oil Immersion Microscopy

- Different refractive indexes for water/air.
- Light bends, loss of resolution, distortion
- Special oil-immersion lens used
- Drop of oil to cover slip

Oil Immersion Microscopy

- Dry lens focus first
- Oil immersion lens next
- Lens nearly touches cover slip when focusing – look from side
- Use w/ very thin specimens

Oil Immersion



With Oil Without Oil

Objective Lens Selection

- Achromatic
  - Color Correction
  - All lens of this type have curvature
- Plan Achromatic
  - Color correction
  - Flatter field of view
- Check & clean all objectives often

Microscope Setup

- Interpupillary adjustments
- Diopter adjustments
- Substage adjustments
- Phase Contrast Condenser alignment
- Phase Contrast Annular Ring alignment

Interpupillary/Diopter Adjustments

- Interpupillary(distance between eyepieces) made thru "folding action" (see fig #2)
- Easily done for each user
- Diopter (corrected vision) for each eye
- Recommended & easily made
- **Note:** *Following adjustments may vary from manufacturer to manufacturer - follow your instrument instructions*

Interpupillary Adjustments

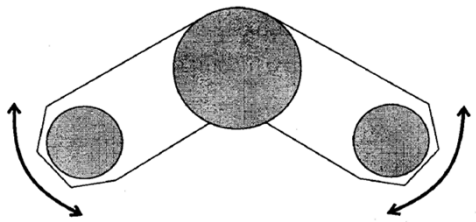
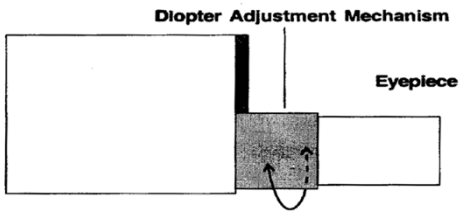


Figure 2

Diopter Adjustments

- 40x objective & sample slide mounted on stage
- Close left eye, focus image w/ right eye using *course/fine* focus control
- Close right eye - check focus with left eye
- Fine adjust with Diopter on left eyetube

Diopter Adjustments



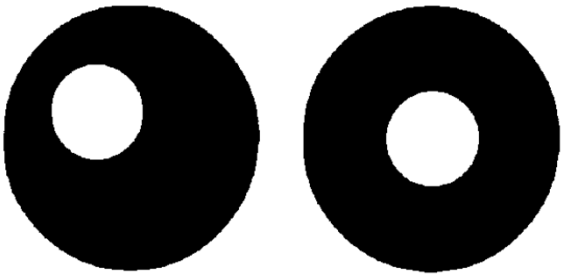
Substage Adjustments

- Centering
- Vertical Focusing
- Aperture adjustments

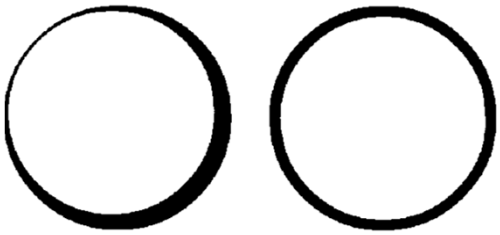
Centering Adjustments(1)

- Start w/ 4X objective in light path
- Looking into eyepieces, close Iris to its smallest setting (*Iris Diaphragm lever*)
- Should see a reduced field w/ small white circle within black field
- Center white circle by tightening & loosening centering knobs
- Fine-tune by opening Iris, white circle nearly fills field - readjust centering knobs

Substage Alignment-Course



Substage Alignment-Fine



Substage adjustments (2)

- Vertical focusing:
  - Condenser raised and lowered w/ *Substage adjustment knob* to focus light
- Aperture Adjustments:
  - Light path adjusted w/ *Iris lever* to introduce contrast into specimen viewing - not to adjust light intensity.

Phase Contrast Adjustment

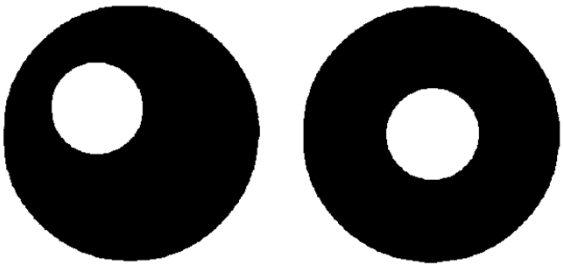
Phase Contrast Condenser Centering(1)

- Must be centered in light path
- Illumination on, 100X objective, sample slide mounted on substage
- Use *course/fine* adjust to bring tip of the 100X just above slide w/o touching
- Rotate 10X objective into light path
- Lower Phase Contrast condenser assy 1/2 way down using substage adjustment

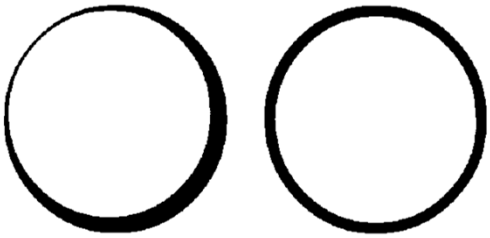
Phase Contrast Condenser Centering (2)

- Rotate *Annuli Turret* in condenser to "0"
- Close Iris to its smallest setting (*Iris Adj lever*)
- Look for small white circle within black field
- Center by using *condenser centering knobs*
- Fine tune by opening Iris completely, and readjust large white circle w/in black field using *condenser centering knobs*

Align Phase Contrast Condenser-Course



Align Phase Contrast Condenser-Fine



Phase Contrast Annular Ring Centering(1)

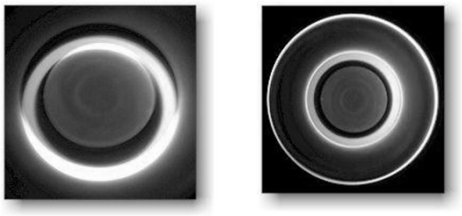
- Phase Contrast Condenser must be centered before proceeding
- Illumination on, 100X objective, sample slide in place, set objective close to slide without touching (*course/fine adjustment*)
- Rotate 10X objective into light path
- Rotate *Annuli Turret* to "10" position
- Remove eyepiece from one eyetube

Phase Contrast Annular Ring Centering(2)

- Install *Phase Contrast Centering Telescope* into eyepiece
- Look through centering telescope and focus using uppermost piece
- Image seen should resemble series of rings superimposed on one-another
- Adjust *Phase Annuli Centering knob* to center two rings upon one-another
- Reinstall original eyepiece -- ready to use!

Centering Telescope

Phase Contrast Annular Ring Alignments



Scope Care & Maintenance

- Never touch lens!
- Never leave slide on stage when not in use!
- Always remove oil from objective
- Stage should be kept clean

Scope Care & Maintenance

- Do not tilt Microscope when using oil
- Keep Microscope covered when not in use
- Regular professional service

Tips & Tools

- Never force mechanical components, adjustments should work freely
- Don't allow lens to touch slide
- Never interchange different mfr. lens

Sample Collection(1)

Potential sampling areas

- Aeration Basin Mixed Liquor
- WAS
- RAS
- Wastewater Inf. / Eff.

Sample Collection(2)

- 100 mL plastic bottles
- Sample:
  - Mixed liquor near effluent to clarifier
  - Discharge from secondary clarifier center well
  - RAS pump discharge
- Sample foam if suspect nocardia or other foaming organisms

Wet mount Cover Slide Prep, (1)

- Clean slide & cover slip
- Shake sample bottle, transfer 50 mL to beaker
- Drop of sample to slide center
- Hold cover slip at 45 deg above sample
- Slide slip toward sample drop
- Allow sample to spread to cover slip edge

Wet mount Cover slide Prep, (2)

- Drop slip into place on sample
- Gently press slip w/ pencil eraser or Q-Tip to spread
- Absorb excess sample with tissue
- ID the slide with appropriate markings

Cover Slide Preparation

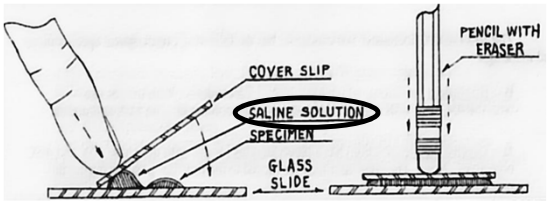
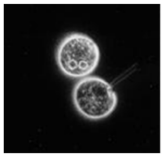
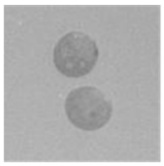


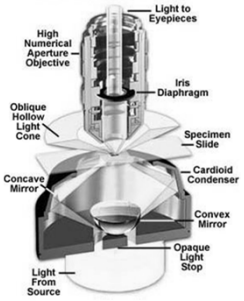
Diagram for light usage

Darkfield

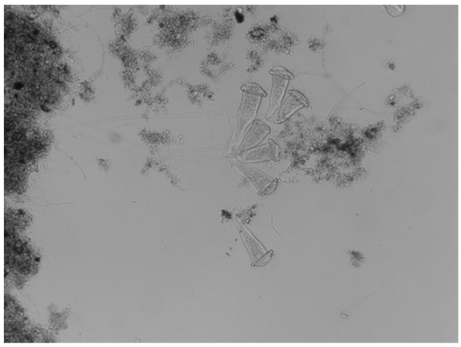


Brightfield

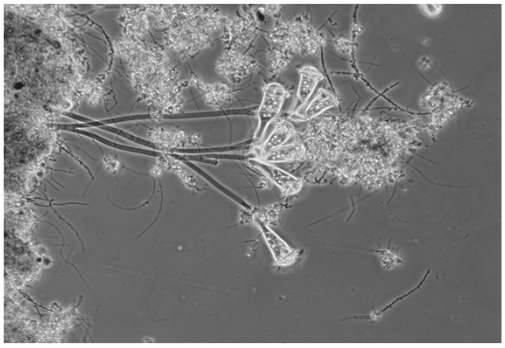




Brightfield View 100X



Phase contrast View 100X



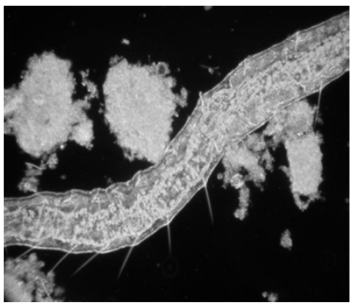
Brightfield

- Uses include:
  - Stained samples
  - Blood samples
  - Pathogens
  - Water sample

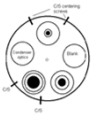
Light from a lamp is concentrated and directed onto the specimen. Most bacterial cells are difficult to see because of their lack of contrast from the surrounding medium.

Darkfield Usage

- Uses include:
  - Density tests
  - Inorganic (filter)

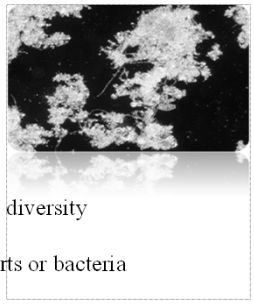


Phase contrast Condenser



Daily Microexams (on site)

- Should be performed daily or at least 3X per week
- Limited in scope
  - **F**ind **F**unny **H**ungry **B**ugs
    - Floc
      - Size, shape, density
    - Filaments
      - Abundance, type
    - Higher life forms
      - Presence, abundance, diversity
    - Bulk water
      - Clean, containing inerts or bacteria



Typical Sampling

- Collect in screw-cap, plastic sample bottles.
  - Small amount of sample is needed (< 100 ml).
  - Fill bottle only half full.
- Observe shortly after collection for on-site analysis.
  - Refrigerate and ship on ice for off-site analysis.
- Sample mixed liquor from the effluent side of tank.
  - If parallel streams, analyze sample from each stream.
- If foam occurs, collect mixed liquor without foam contamination.
- If foam is to be analyzed, collect in screw cap bottle without mixed liquor contamination.

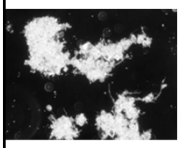
Performing the exam

- Place small drop on slide (3 if possible)
- Place glass coverslip on top (not plastic)
- Place slide & coverslip between paper and lightly pat to remove excess water* (nail polish can seal coverslip)
- Observe under 10X magnification (provides wider view)
- Increase magnification to 40X (change condenser setting)
- If possible observe under 100X magnification (remember only this magnification requires oil immersion)
- Make notes and draw pictures*
- Clean any oil off objectives immediately
- Place dust cover on microscope

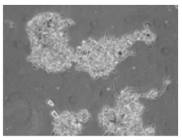
Organic Loading and Predominant Microlife

Condition	Predominant Groups
High F/M ; low MCRT	Flagellates, amoeba, small free-swimming ciliates
Moderate F/M ; average MCRT	Good diversity. Dominated by free-swimming and stalked ciliates
Low F/M ; high MCRT	Stalked ciliates, rotifers, and higher invertebrates.

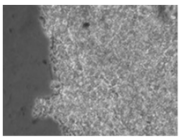
Objective Progression



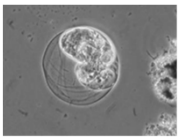
10X darkfield



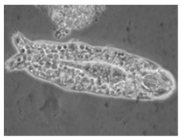
10X Phase contrast



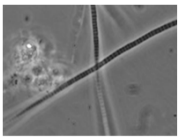
40X Phase contrast



40X Phase contrast



40X Phase contrast



100X Phase contrast w/oil immersion

Slide Staining

- Adds to organism visibility/contrast
- More organism detail
- May help low-featured microscope performance.
- Enhance Bright Microscopy and those microscopes w/o Phase Contrast.
- But staining usually kills organism
- Some stains require ‘smear’ slide preparation

Slide Prep, Staining[1]

- Clean slide & cover glass
- Drop of sample in center of slide
- Spread/smear sample w/ glass rod
- Air-dry (do not use a heat source…hair-dryer, lighter…)
- Stain per Standard Methods, following protocol, or manufacturer instructions

Staining Types

- Gram stain
- Neisser stain
- India Ink reverse stain
- Polyhydroxybutyrate (PHB) stain
- Crystal Violet Sheath stain

Gram Stains

- Available as kit from lab supply houses includes:
  - Gentian Violet solution
  - Crystal Violet solution
  - Gram's Iodine solution
  - Decolorizer
  - Safranin solution

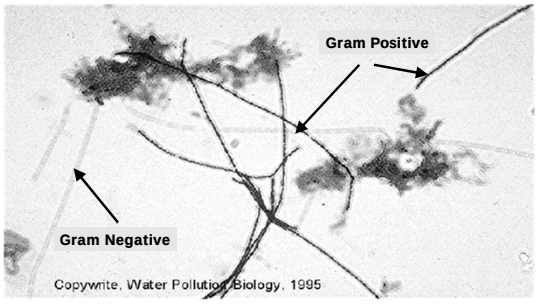
Gram Stains How-to(1)

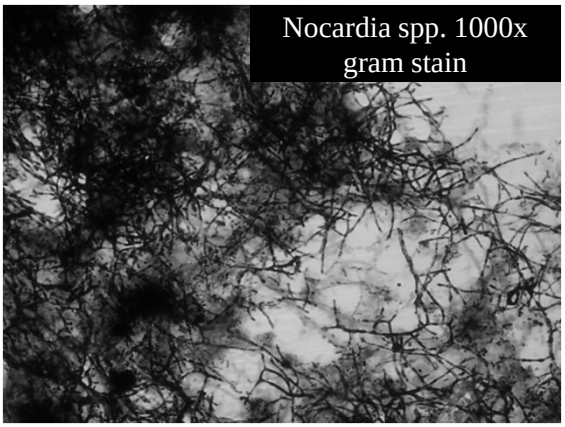
- Prepare thin smear of sample-air dry
- Stain 1 minute w/ Gentian Violet - rinse 1 sec in water
- Stain 1 minute w/ Gram's Iodine solution, rinse well
- add Decolorizing agent drop-by-drop for 25 seconds, Blot dry

Gram Stain, How-To(2)

- Stain w/ Safranin Solution for 1 minute
- Examine using 1000X under oil immersion, *no phase contrast*
  - Blue-Violet is Positive
  - Pink-Red is Negative

Filamentous Organism, Gram Negative & Positive

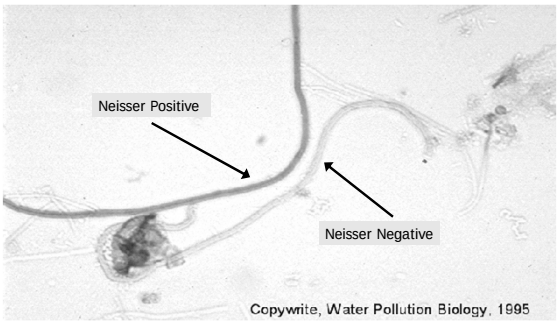


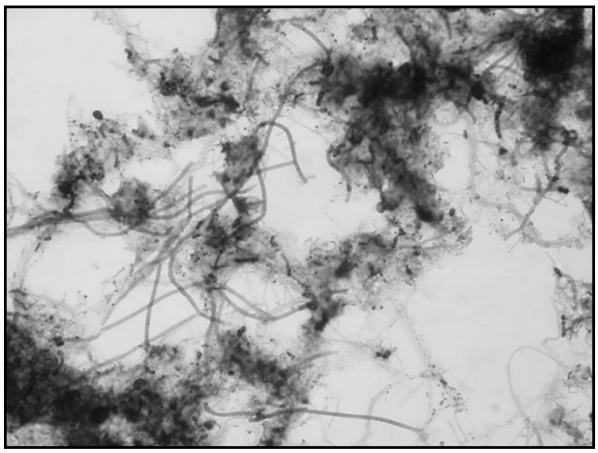


Neisser Stain How-to

- Prepare as required via Standard Methods or purchase from supply house
- Prepare thin smear - air dry
- Stain 1 min w/ solution #1, rinse 1 sec in water
- Stain 1 min w/ solution #2, rinse well w/water, blot dry
- Examine @1000X Oil immersion, *no phase contrast*:
  - Blue-violet = Positive,
  - Yellow-Brown = Negative

Filamentous Organism, Neisser Negative & Positive





India Ink Reverse Stain, How-to

- Mix 1 drop India Ink w/ one drop Activated Sludge on slide
- Cover slide and view @ 1000X
- Normal: Ink particles penetrate floc completely, small clear center
- Abnormal: Large clear areas w/ low density cells

PHB Stain How-to

- Two solutions:
  - #1Sudan Blk, 0.3% w/v 60% ethanol
  - #2 Safranin 0, 0.5% (supply house)
- Thin smear on slide, air-dry
- Stain 10 min w/ solution # 1 avoid dry-out
- Rinse 1 minute in water
- Stain 10 seconds w/ solution #2, rinse, blot dry
- View @ 1000 Oil immersion, PHB Blu-Blk, Cytoplasm - Pink to clear

Crystal Violet Sheath Stain, How-to

- Mix 1 drop of activated sludge w/ 1 drop of Crystal Violet Solution, cover and view @ 100X, with phase contrast.
- Cells stain deep violet
- Sheaths are clear-pink

Photographic Cataloging

- Inventory known organisms
- Identify unknown organism
- Helps process control decision-making
- HOWEVER…it may be expensive to buy hardware, and requires some expertise to use and store data

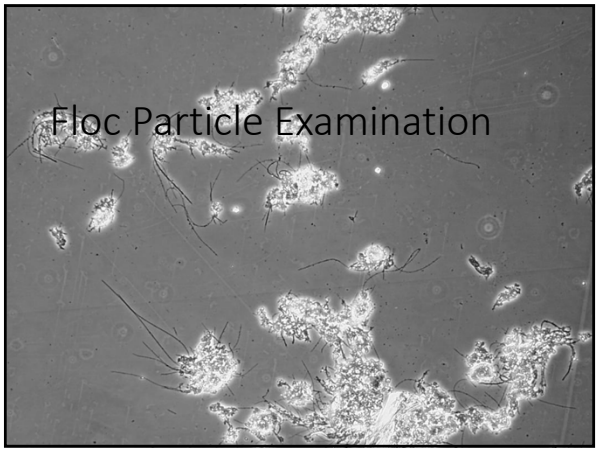
Permanent Slides

- Catalogs known organisms in your plant
- Historical reference data for comparing future slides.
- Helps identify previously unknown creatures.
- Process control tool for decision-making.
- Provides benchmark

Permanent Slide Preparations

- Label Slide
- Use select specimen
- Add drop of Cytoseal next to specimen
- Move cover slip to touch Cytoseal.
- Keep edge of slip down
- Cytoseal will spread across specimen
- Allow to dry, store slide in cool dry location

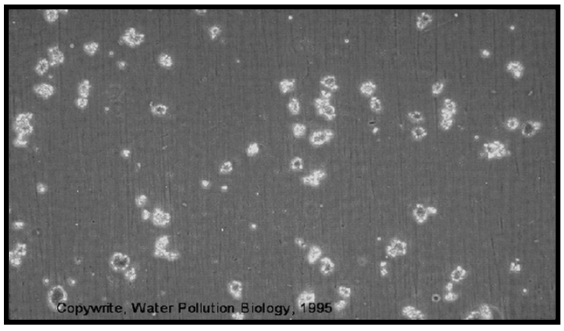
The Bacteria Bowl



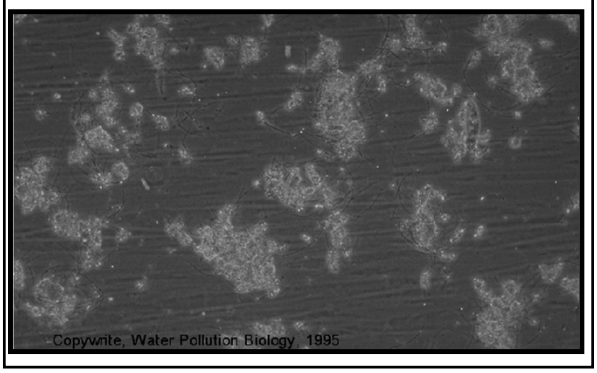
Floc Particle Examination(1)

- Shape
  - Round - immature floc particle
  - Irregular - mature floc particle
  - Oval - congealed, toxic heavy metals
- Dispersed floc - mechanical shearing
  - Irregular shape

Floc Particles, Spherical Shape



Floc Particle, Irregular Shape



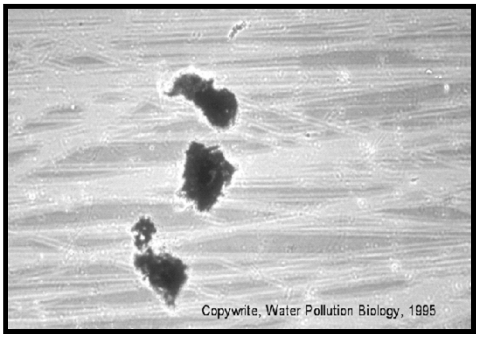
Floc Particle Examination(2)

- Size
  - Small
  - Medium
  - Large
- Strength/Surface area
  - Tightness of bacteria fit (floc particle/openings)

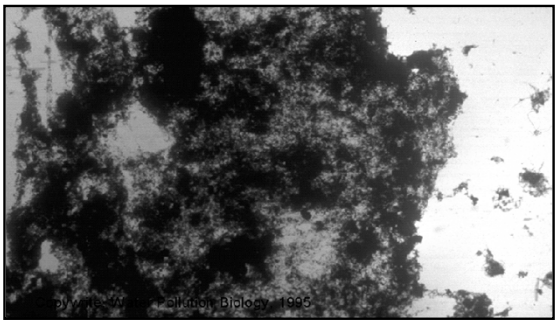
Floc Particle Examination(3)

- Wet mount Mixed Liquor with 1 drop Methylene Blue
- Weak floc
  - loosely compacted, large openings between particles
- Firm floc
  - Tightly compacted, few or small openings

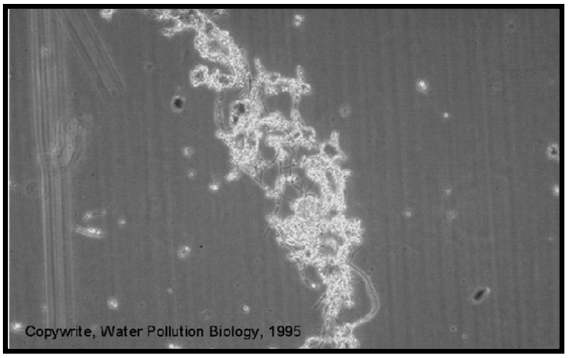
Particle Floc, Firm (Meth/Blue)



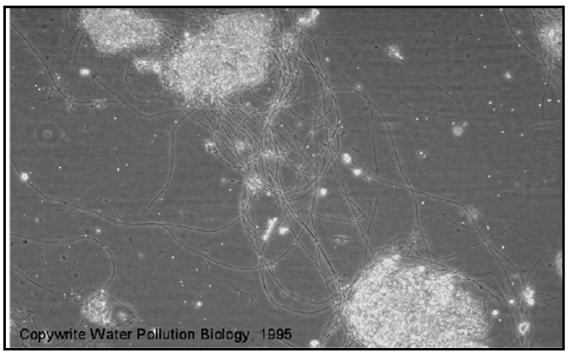
Floc Particle, Weak (Meth/Blue)

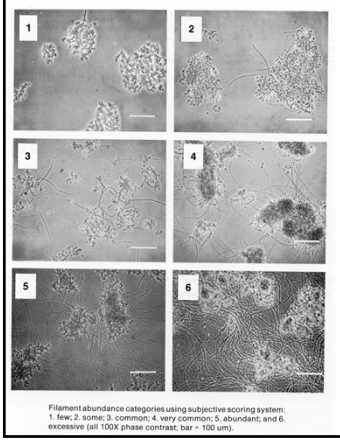


Open & Diffused Floc Formation



InterFloc Bridging





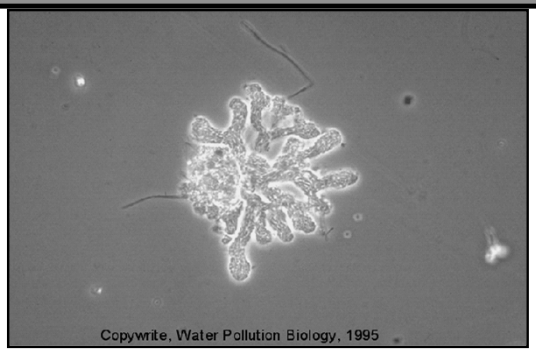
Filament abundance categories using subjective scoring system: 1. few; 2. some; 3. common; 4. very common; 5. abundant; and 6. excessive (all 100X phase contrast; bar = 100 um).

Filament abundance ranking

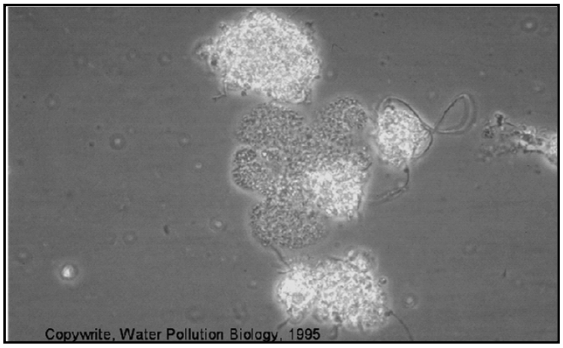
Zoogleal Growth

- Rapid growth of 1st floc-forming bacteria
- Creates weak floc
- Easily sheared
- Lower settleability
- Floc-forming bacteria held loosely together by insoluble gelatinous material
- Floc is less dense
- Both forms recognized under 100 to 400X

Zoogleal Growth, Dendritic, (finger-like)



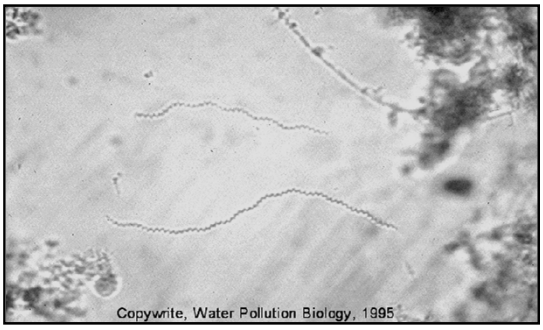
Zoogleal Growth, Amorphous



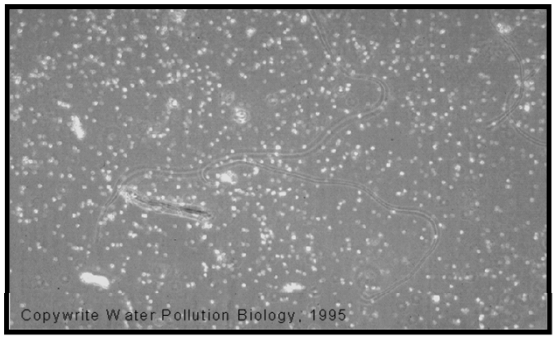
Spirochetes

- Wavy/spiral-shaped
- Very motile bacteria
- Rotates like corkscrew
- Some are pathogenic
- No specific operational function
- More growth associated under anaerobic conditions.

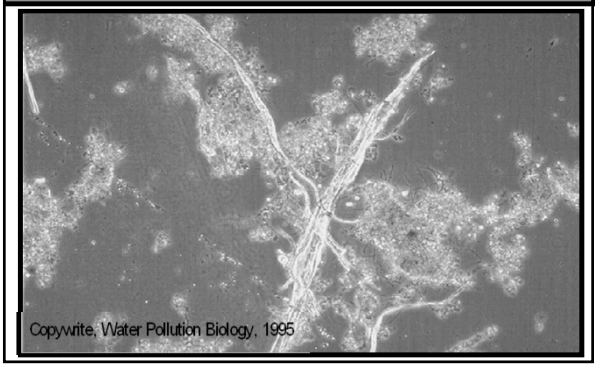
Spirochetes



Particulate, Free-Floating



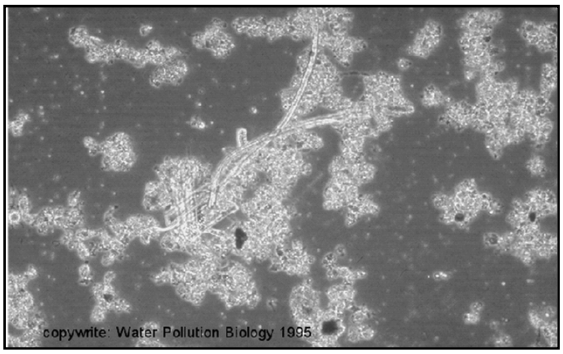
Particulates, Protruding from Floc Particles



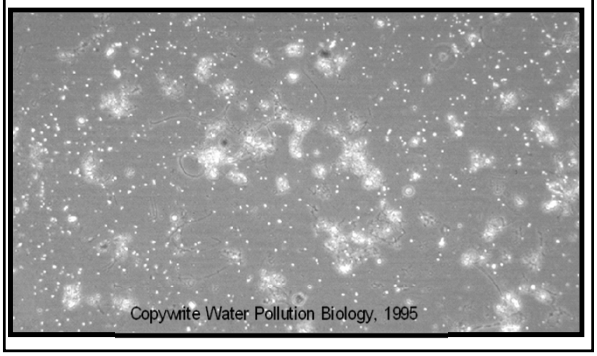
Dispersed Floc Growth

- Wet mount scan a number of samples to see if:
  - Very young sludge
  - Toxic wastes present
  - Shearing / turbulence
  - Lack of active ciliates
  - Low DO

Dispersed Growth, young sludge



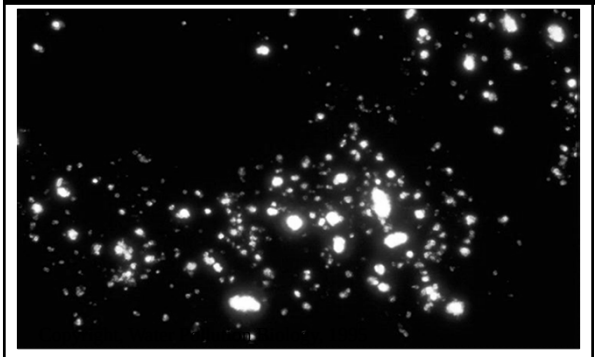
Dispersed Growth, old sludge



Nutrient Deficiency

- CBOD creates insoluble polymeric material
  - Buoyant floc
  - Low Settleability
- Wet mount w/ India Ink stain
  - Large white areas within floc particle
  - Low degree stain penetration, greater nutrient deficiency

India Ink Reverse Stain, Negative



India Ink Reverse Stain, Positive

Nutrient Deficient Wastewater

- Improper ratio of vital nutrients in influent waste stream
  CBOD : N : P : Fe
  (100 : 5 : 1 : 0.5)
- Excessive I&I
- Industrial wastes

Organism Families

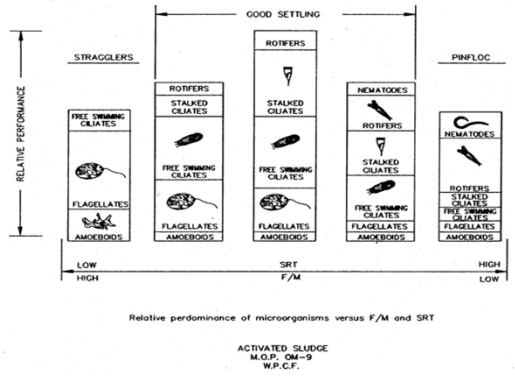
- Bacteria
- Protozoa
  - Amoebae
  - Flagellates
  - Free/Stalked Ciliates
- Metazoa
  - Rotifers
  - Nematodes
  - Tardigrades
- Fungi/Algae

Dominant Group Scans

- Stir Sample
- Prepare three separate wet mount sample
- Scan each slide with Bright Field, drop of Methylene Blue
- Try Darkfield microscopy here as well
- Determine dominant groups of each slide
- Record on worksheet for each group of organisms.

PROTOZOAN FAMILY

Abundant & Diverse in the Activated Sludge process



Protozoan Activity Scans

- Under toxic conditions activities decrease
- Scan wet mount w/ “suspect” sample
- Compare with “normal” activity scan
- Inhibitory/toxics result in sluggish activity levels
- Compare/contrast via 100X or high magnification

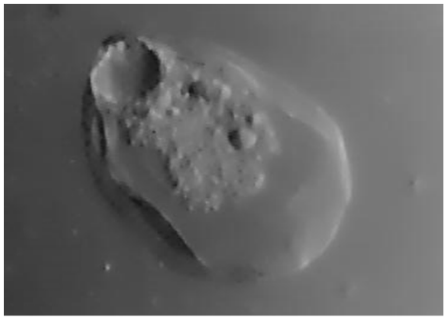
Protozoa

- Inactive?
  - Toxic Shocks?
- No Protozoa?
  - F/M too high (Reduce wasting, incr. return)
  - Low to normal F/M (Incr. DO, toxic shock)
- Healthy Protozoa, Dispersed Floc?
  - Reduce mixing, reduce aeration

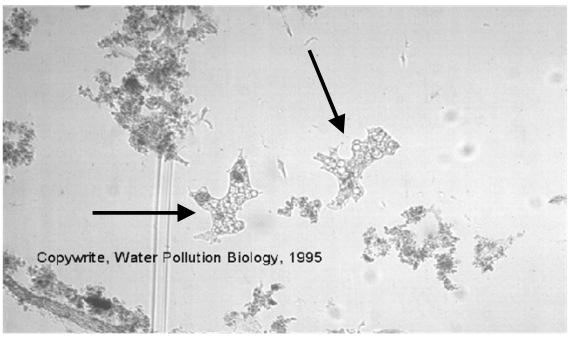
Amoebae

- Earliest protozoan that appears in Activated Sludge process
- Associated with “young sludge” Feed by Pseudopodia (False Feet)
- Engulf small organic matter.
- Can be difficult to see w/ Bright Field microscope

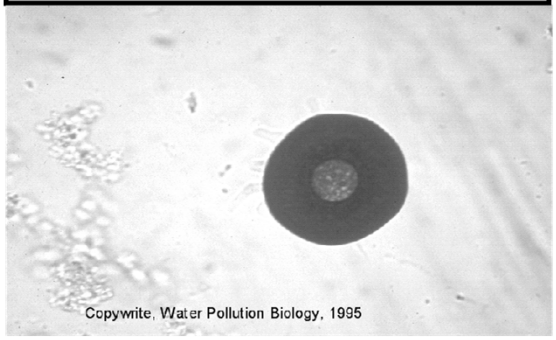
Amoebae



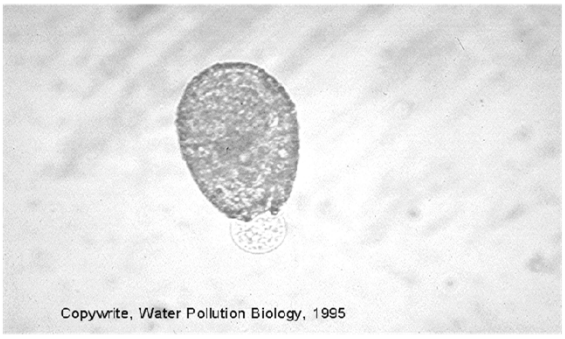
Amoebae, *Amoebae proteus*



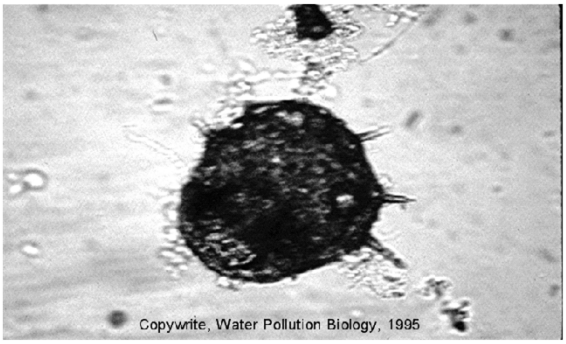
Amoeba, *Arcella*



Amoeba, *Difflugia*



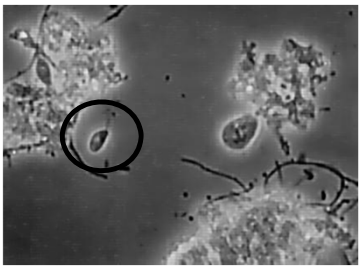
Amoeba, *Centropysis*



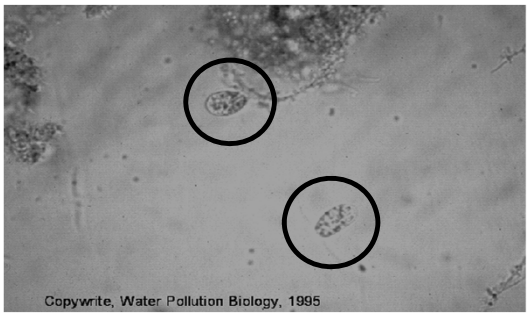
Flagellates

- Tail-like structure which whips back & forth for motility.
- Engulf organic particles & bacteria
- Can be seen in Bright Field microscope with careful magnification and cell staining.
- Some are very fast moving

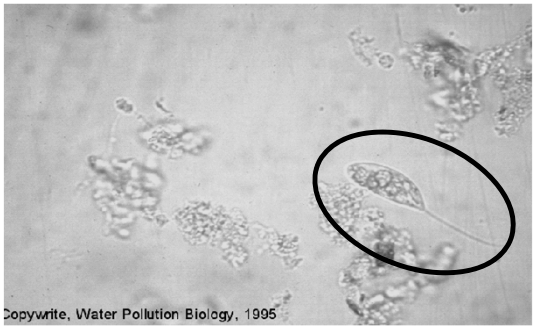
Flagellate



Flagellate, *Paranema*



Flagellates, *Bodo*



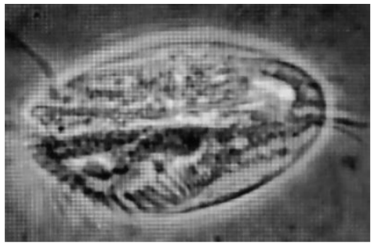
Free & Stalked Ciliates

- Highly prized in most WWTP
- Associated w/ good settleability
- Low suspended solids
- Organism in sweeping motion

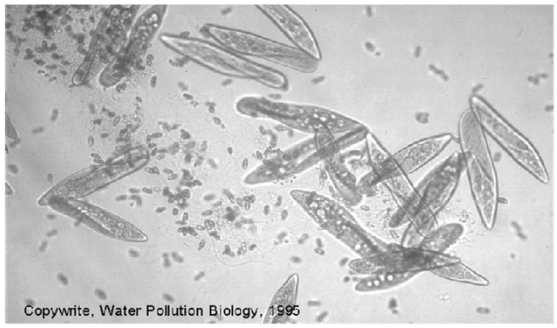
Free & Stalked Ciliates

- Sweeping effect by ciliates gather small particles and help form floc
- Large floc particles settle well
- Requires good Bright Microscopy to see these organisms.
- Phase Contrast may offer better visibility

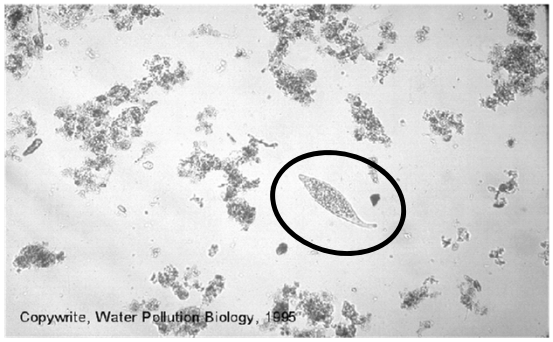
Free & Crawling Ciliates



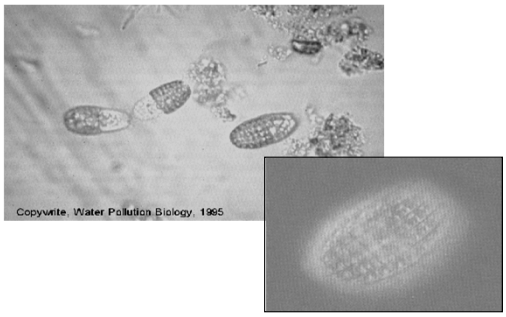
Free-Swimming Ciliate, *Paramecium*



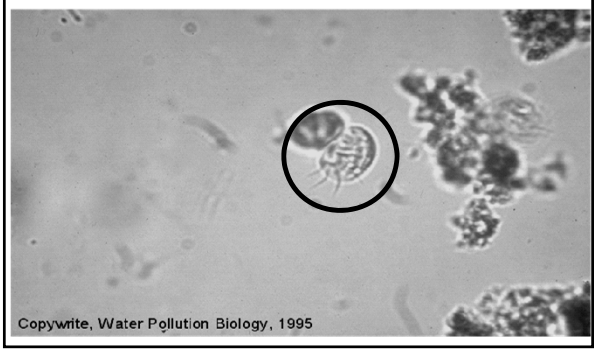
Free-swimming Ciliate, *Litonotus*



Free-swimming Ciliate, *Coleps*

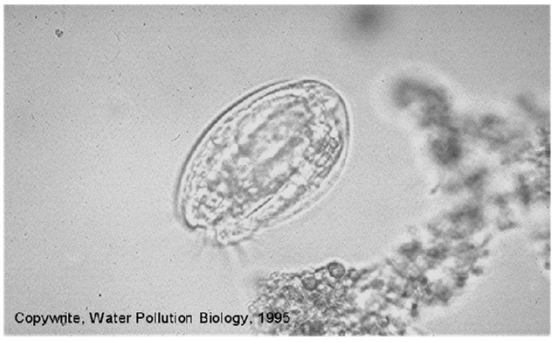


Crawling Ciliate, *Aspidisca*

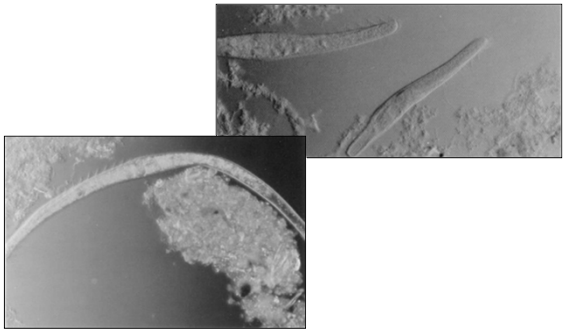


Crawling Ciliate, *Aspidisca*

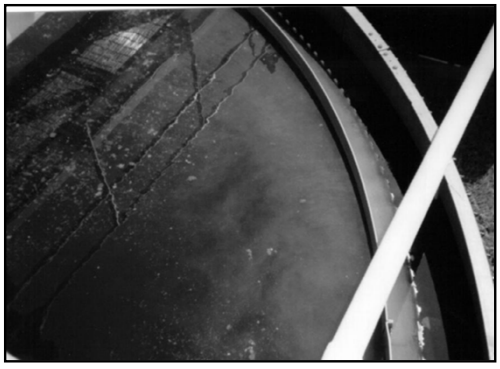
Crawling Ciliate, *Leptopharynx*



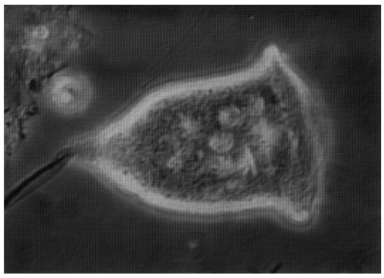
Spirostomum



Spirostomum

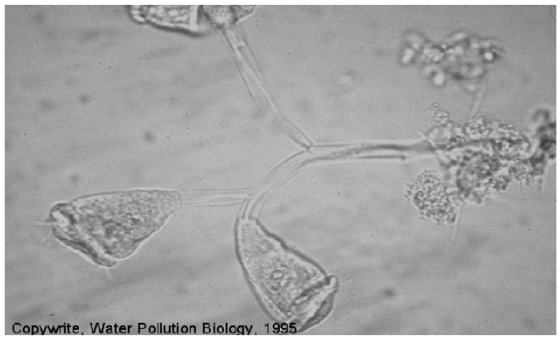


Stalked Ciliates

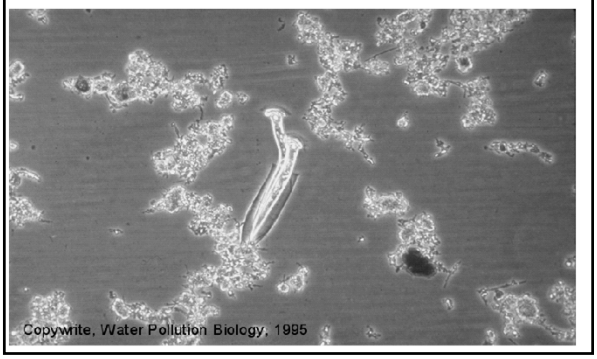


Stalked Ciliate, *Vorticella*

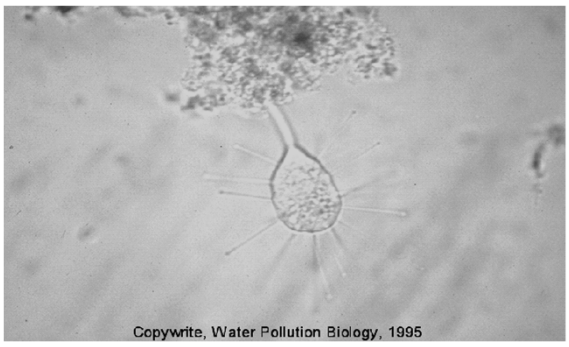
Stalked Ciliate, *Carchesium*



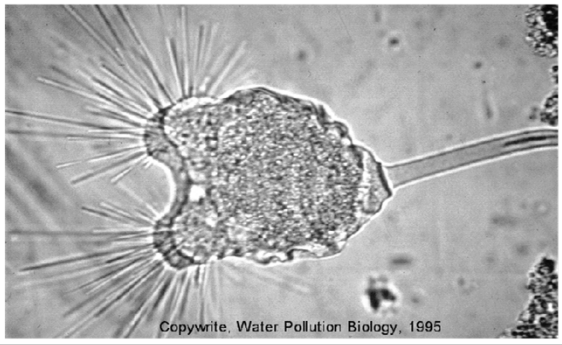
Stalked Ciliate, *Vaginicola*



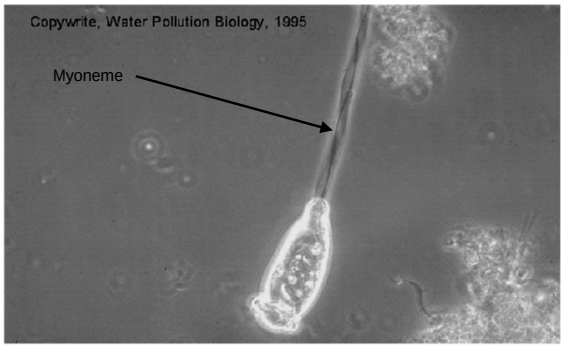
Suctorian, Tentacle Stalk



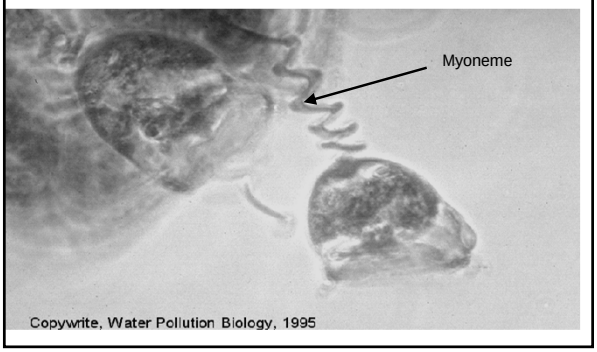
Suctorian, Enlarged Anterior Portion



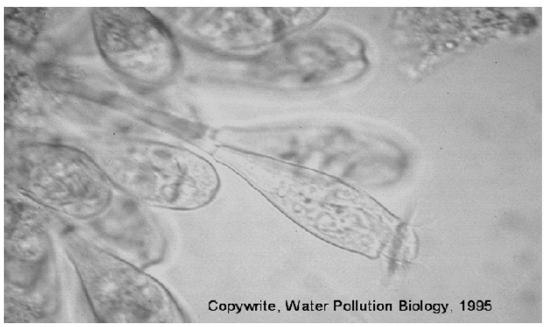
Stalked Ciliate, *Myoneme*



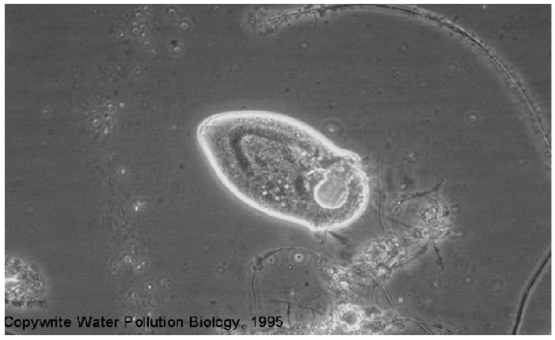
Stalked Ciliate, Myoneme Methylene Blue Stain



Stalked Ciliate, Anterior Cirri & Absence of Myoneme

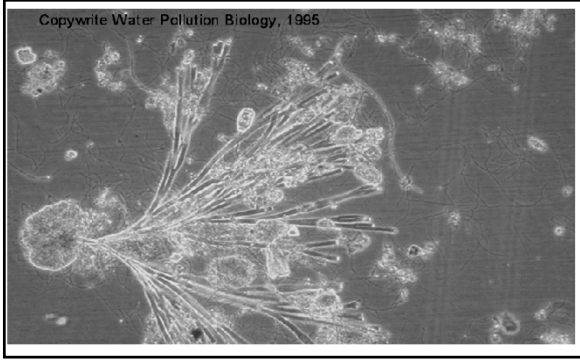


Stalked Ciliate, Sheared Anterior

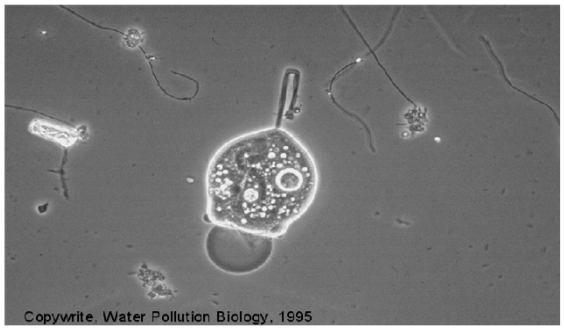


Stalked Ciliate, Sheared Posterior

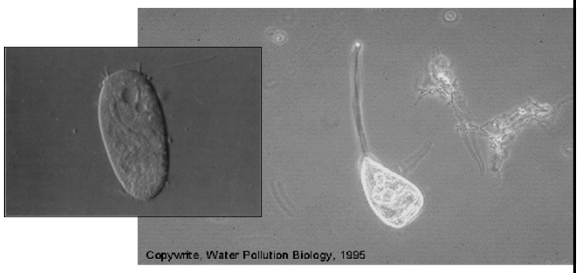
Stalked Ciliate, Sheared Colony



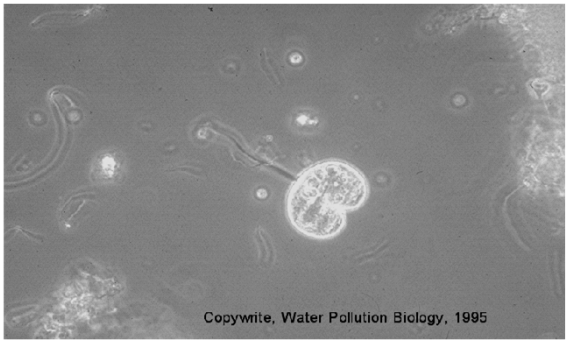
Stalked Ciliate, “Bubble” Produced Under Toxicity



Stalked Ciliate, Free-swimming Mode



Stalked Ciliate, Dividing Asexually



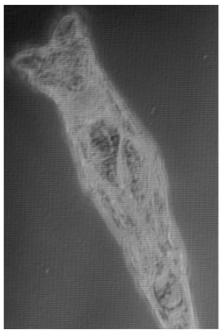
Rotifers

- Rotary cilia sweep small particles into mouth.
- Constantly moving
- Some wastewater operators may prefer larger numbers in Activated Sludge process
- They help promote better settling by consuming floc bacteria and secreting enzymes

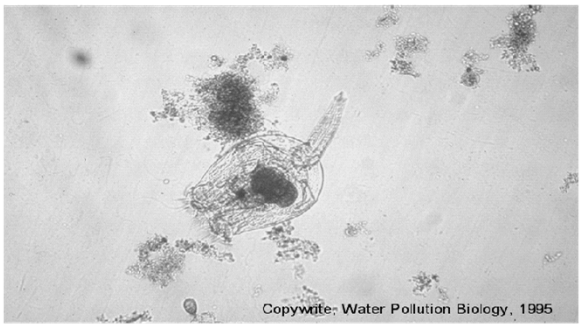
Rotifers

- At some wastewater plants, higher numbers mean old sludge and more wasting is needed
- Best viewed with Brightfield Microscopy at 300X to 400X power

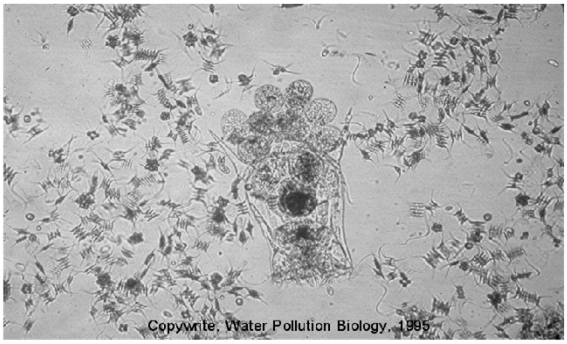
Rotifers



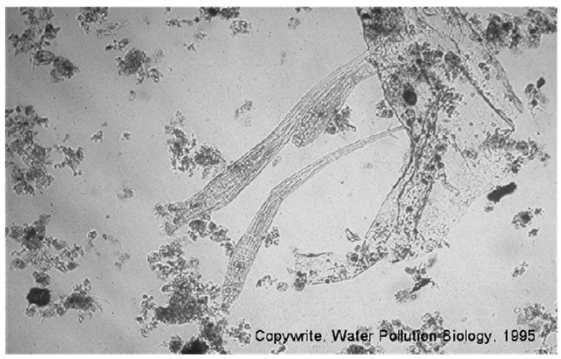
Rotifer, *Brachious*



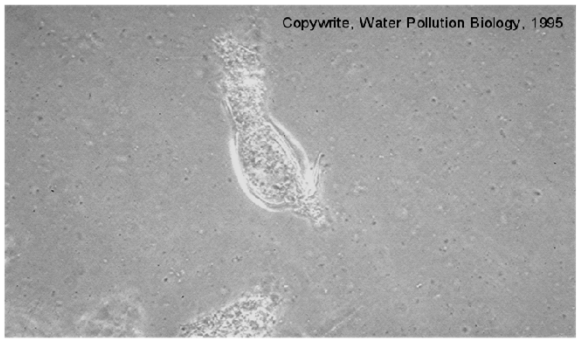
Rotifer, *Brachious*, Female w/ Eggs



Rotifer, *Philodina* (extended)



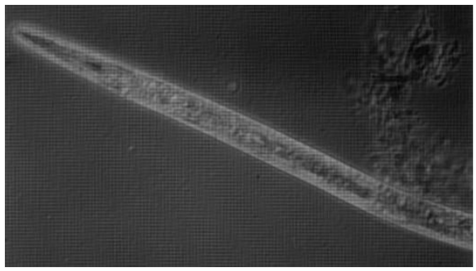
Rotifer, “Horseshoe” Under Cell Bursting Agent



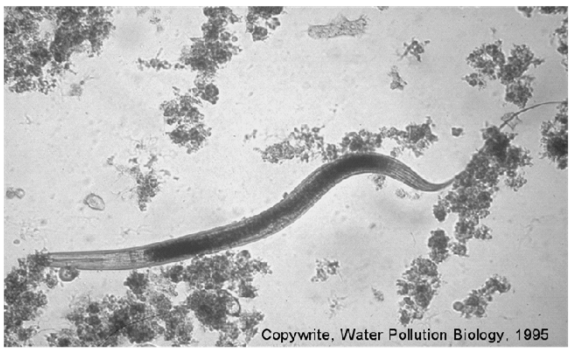
Nematodes

- Roundworms that feed on organic matter and bacteria.
- Associated with an old sludge
- Substantial numbers usually a sign to increase wasting rates.
- Some are predators feeding upon protozoa, rotifers.
- Best viewed upon Brightfiels Microscopy @ 100 to 400x

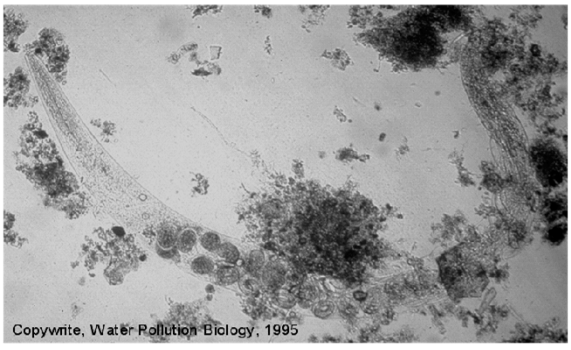
Nematodes



Free-living Nematode

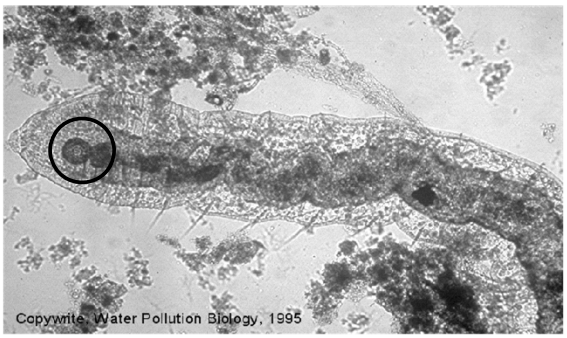


Free-living Nematode, Female w/ Eggs

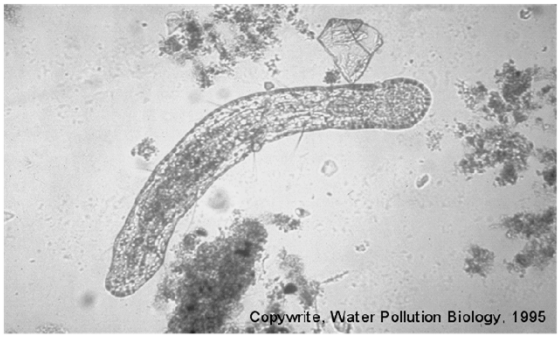


Other Metazoa

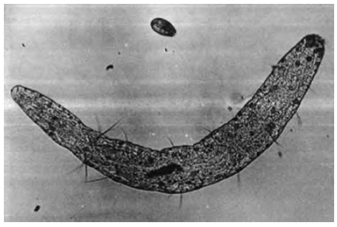
Bristleworm, (*Oligochaete*), ingesting *Arcella*



Bristleworm, *Aeolosoma*

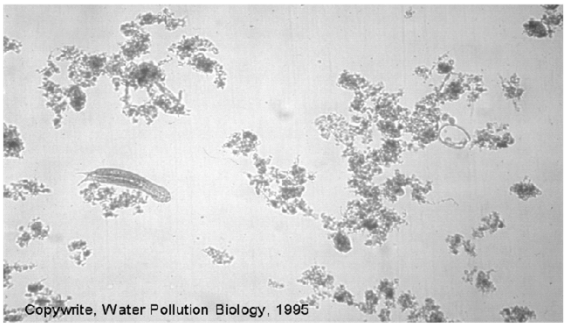


Bristleworm, *Aeolosoma*

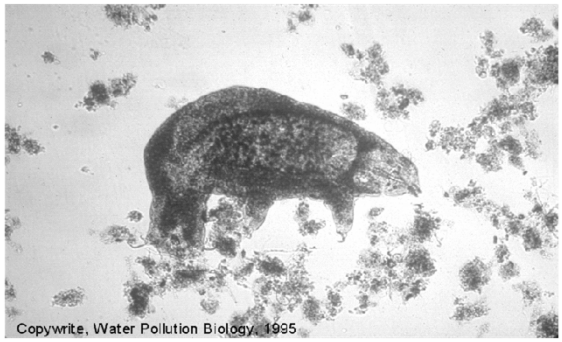


Flatworm

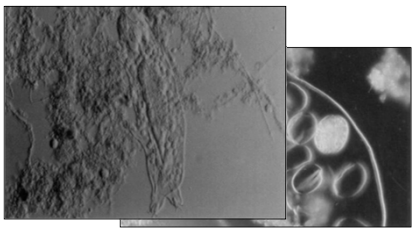
Gastrotrich, (note forked tail)



Waterbear, *Tardigrade*



Waterbear, *Tardigrade*



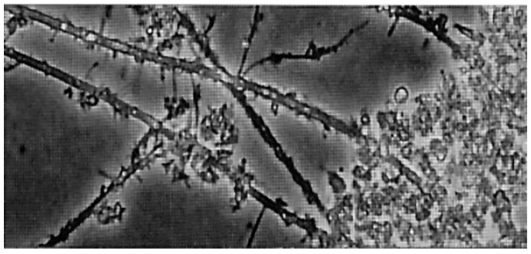
Bacteria

- Convert dissolved organic material
  - Phosphates
  - Sugars
  - Proteins
  - Starches
- Protozoa present as well
- Poorly visible w/ Brightfield Microscopy
- Filamentous visible here
- Requires Phase Contrast to view individual sizes and shapes

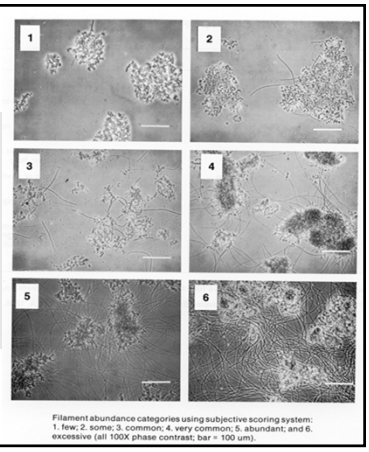
Filaments

- Some Filaments OK for floc formation
- Excess?:
  - Check DO levels > 1 PPM
  - Nutrients (C, N, P, FE)
  - pH
- No Filaments?
  - Check F/M Ratio
  - Check DO, reduce if > 3.0 PPM

Filamentous Bacteria

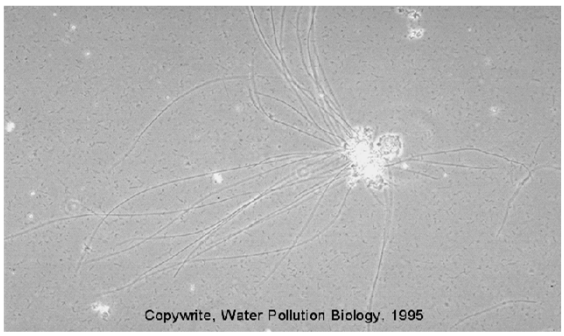


Filament abundance ranking

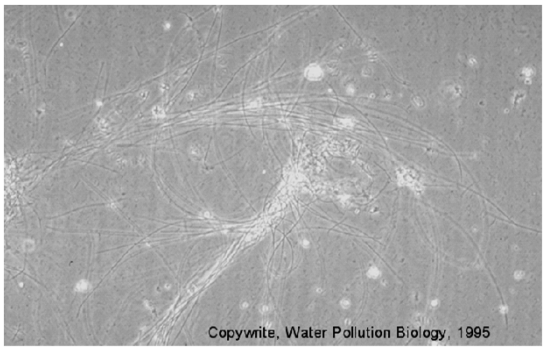


Filament abundance categories using subjective scoring system: 1. few; 2. some; 3. common; 4. very common; 5. abundant; and 6. excessive (all 100X phase contrast; bar = 100 um).

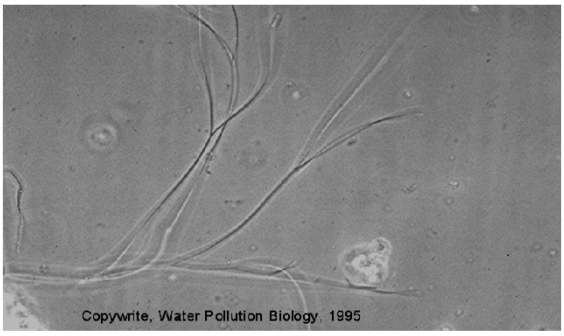
Filamentous Organism, Extending From Floc Particle

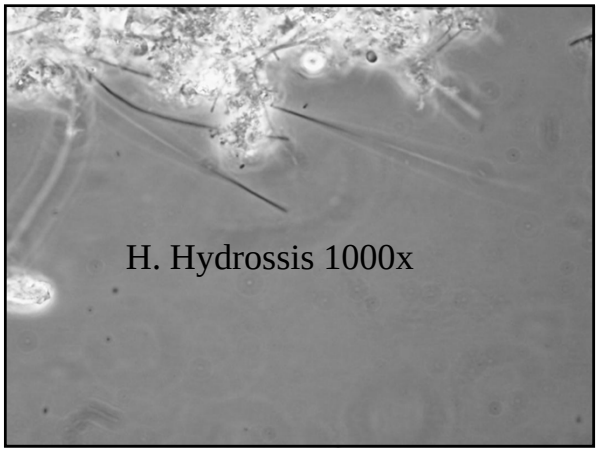


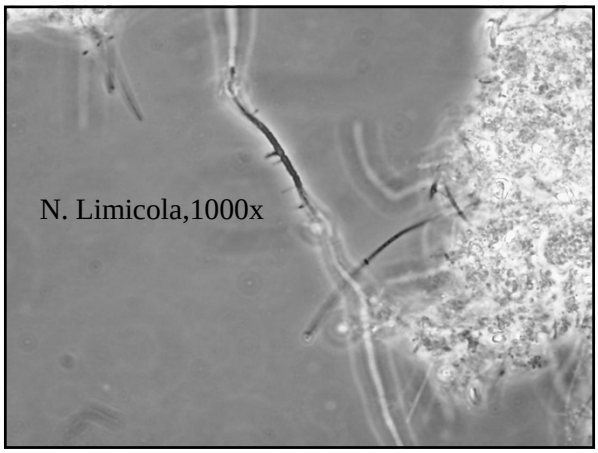
Filamentous Organism, Free-floating

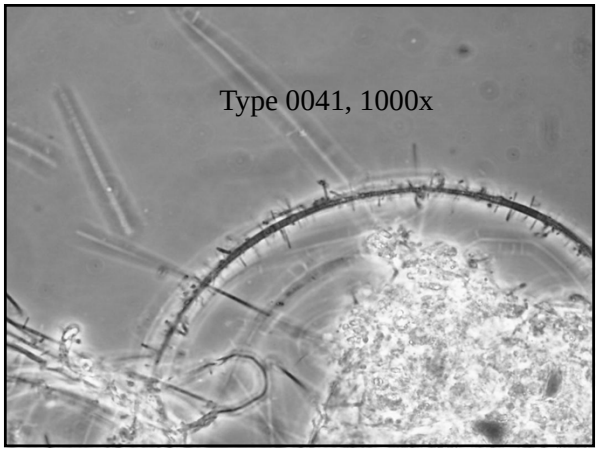


Filamentous Organism, Bacteria; *Sphaerotilus Natans*






N. Limicola,1000x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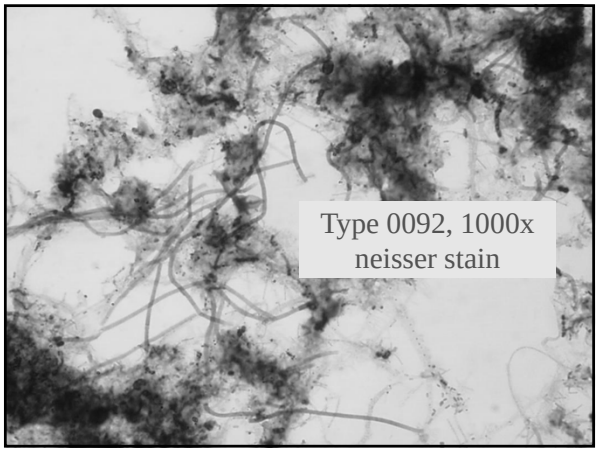
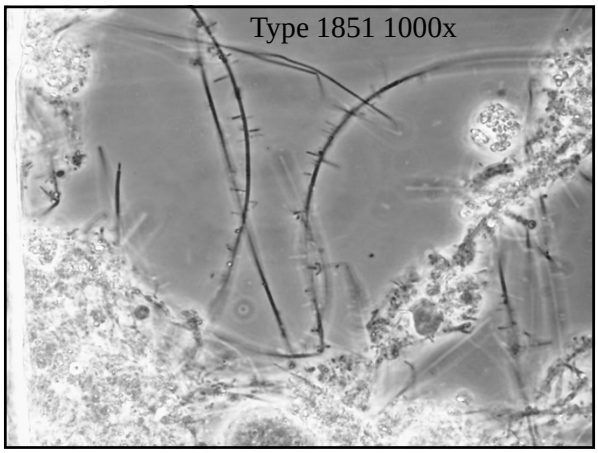
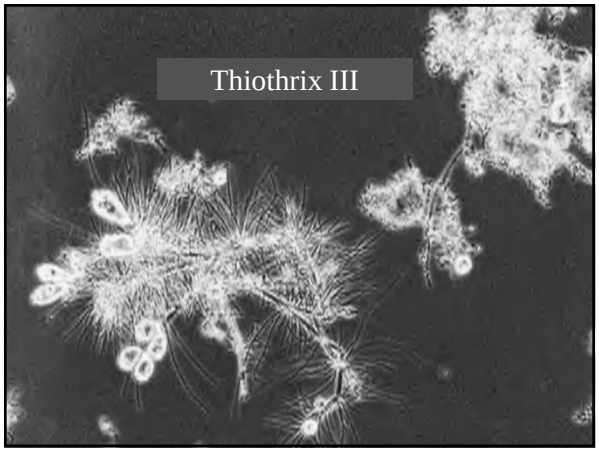
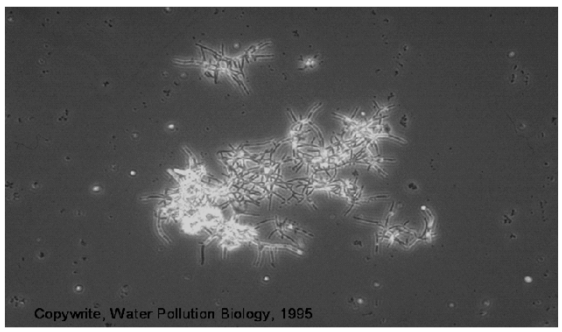

Type 0092, 1000x
neisser stain

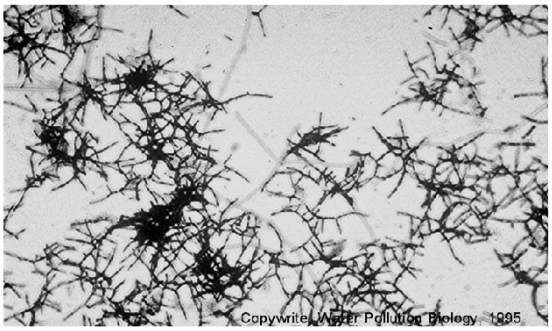




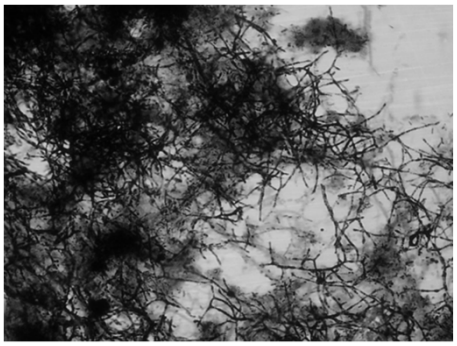
Actinomycetes: Nocardia (wet mount)



Actinomycetes; Nocardia (Gram Stain)



Nocardia (Gram Stain)



Activated Sludge Foaming

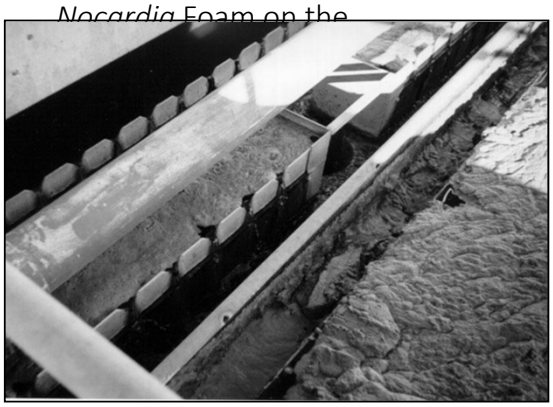
- White light/loose foam may indicate surfactants or young sludge
- White heavy foam - nutrient deficiency
- Heavy-chocolate/brown foam - presence of *Nocardia*
- Dark brown/black foams - anaerobic conditions setting in

Nocardia Foam on the Surface of Aeration Basin

Nocardia Foam on the Surface of Aeration Basin

Nocardia Foam on the Surface of a Clarifier

Nocardia Foam on the



Common Lagoon Filaments

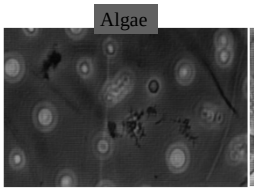
- Most don't cause bulking problems
- Identifying helps define condition of lagoon
- Low DO Filaments
  - *S. Natans, 1701*
  - *Hydrossis*
- Septicity
  - *Thiothrix I & II*
  - *Beggiatoa*

Algae/Fungi

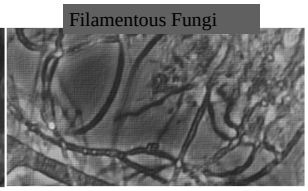
- Common Lagoon or Pond type organisms
- Contribute to Suspended solids
- Add Oxygen in sunlight, Carbon Dioxide in night-time.
- Enhance Phosphorous removal
- Control or harvest is essential.
- Best viewed with Bright Microscopy @ <400X power.
- Phase Contrast helps identify species.

Algae/Fungi

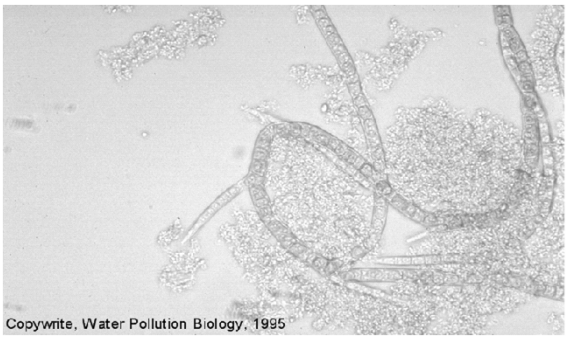
Algae



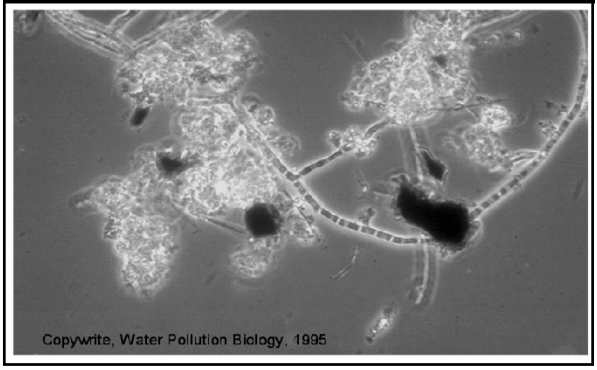
Filamentous Fungi



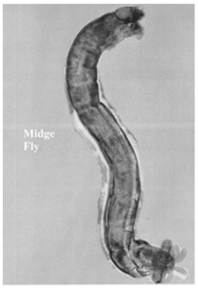
Filamentous Organism, Algae

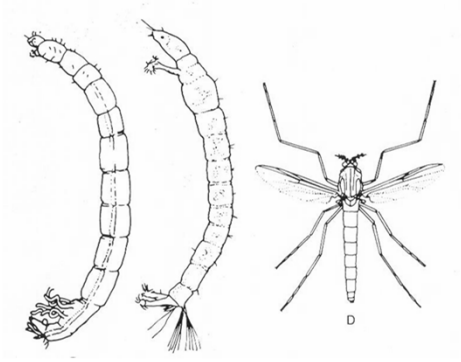


Filamentous Organism, Fungi



Midge Fly Larvae





Midg

Review

- Two Basic Microscope types:
  - Stereo
  - Compound
- Compound best choice for viewing individual specimens and groups

Review

- Choose the best features:
  - Brightfield
  - Oil Immersion Lens
  - Plan Achromatic Lens
  - Phase Contrast
  - 1000X power
- Wastewater Bugs
  - Protozoa
    - Ciliates
    - Flagellate
    - Stalk Ciliates
    - Amoebae
  - Algae/Fungi
  - Bacteria
  - Nematodes
  - Rotifers
  - Filaments

Review

- Sample, prep slides, stain as required and view
- Catalog organisms
  - Type
  - Approx. population
  - Motility
- With other operational tools
  - Activated sludge process control
  - Lagoon process control

Review

- Maintain historical file for future reference:
  - Permanent Slide inventory
  - Photographic file
  - Catalog by type for your process

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