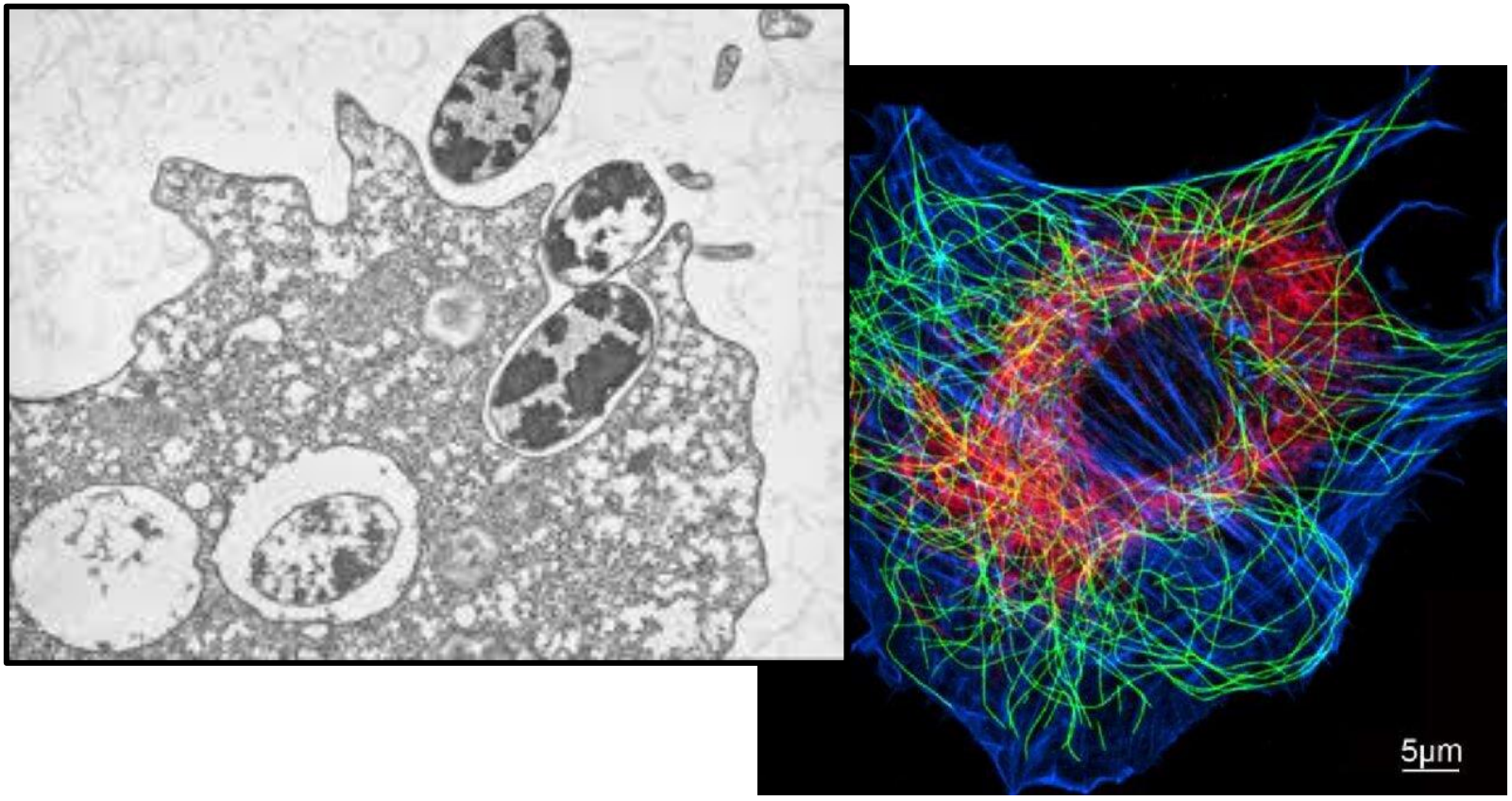
Tutorial 3: Microscopic Examination of the Cytoskeleton



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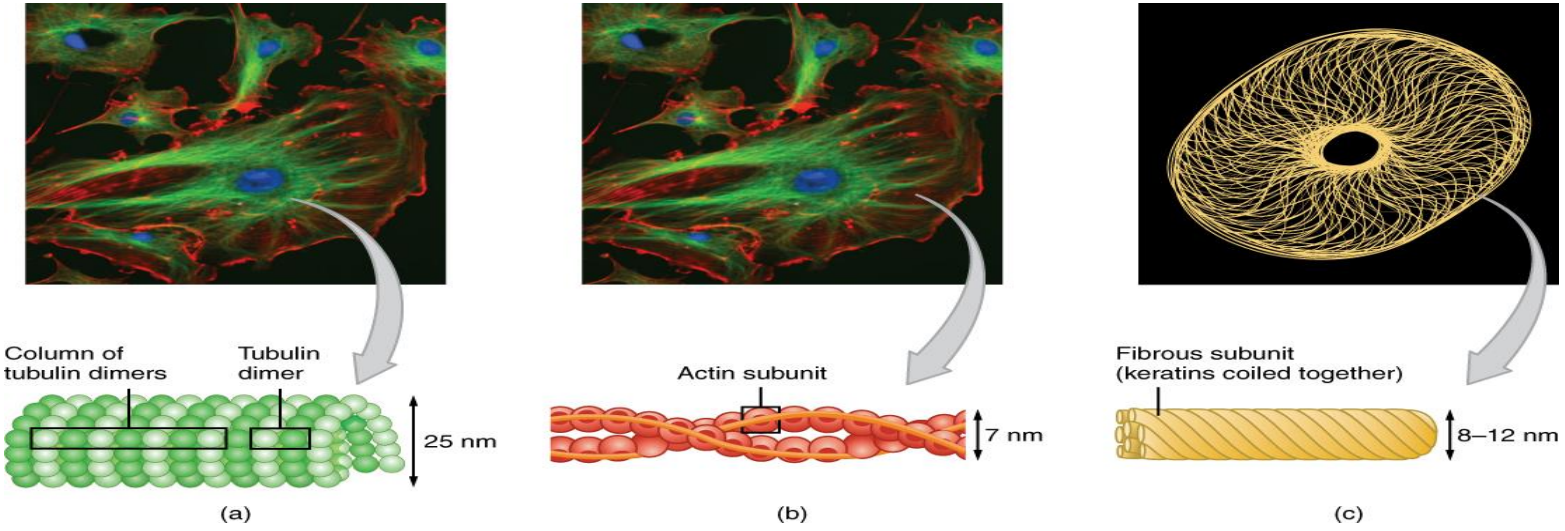
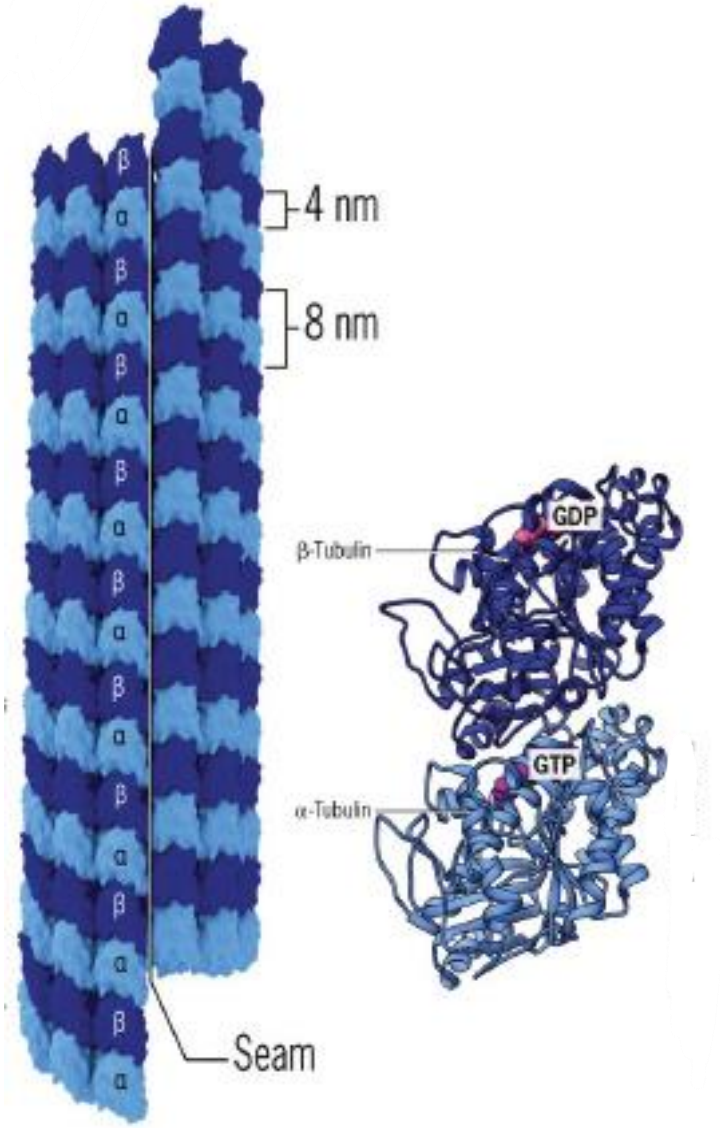
The Cytoskeleton

- Composed of three filamentous structures: microtubules, microfilaments, and intermediate filaments
- Cytoskeletal filaments found largely within the cytoplasm
- Plays a variety of roles in cells:
  - Structural support
  - Transport of intracellular material
  - Cell motility



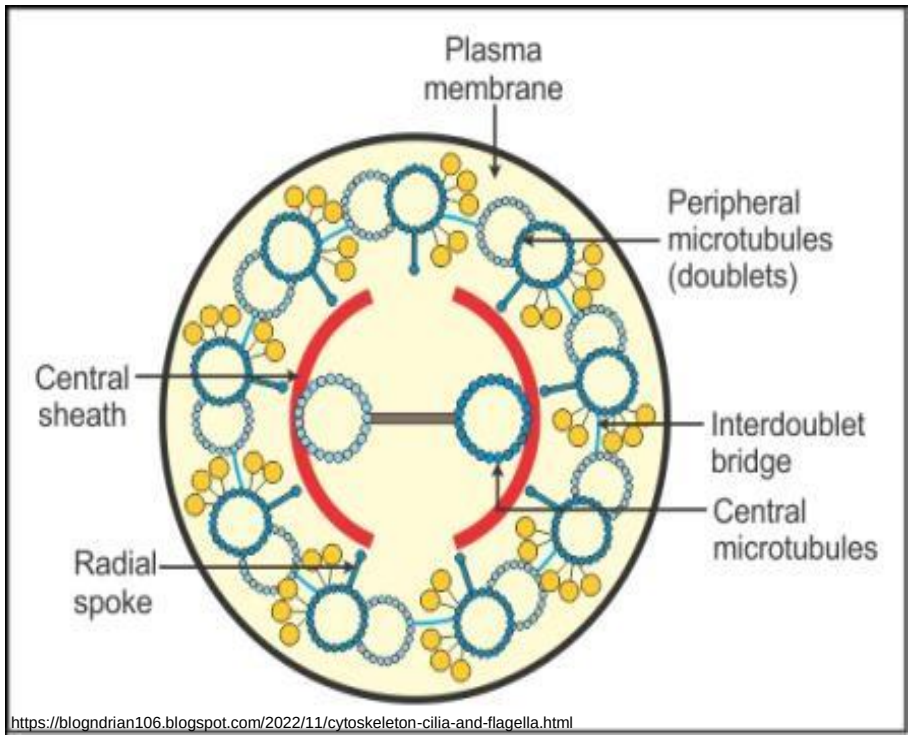
Microtubules

- Hollow, tubular structures composed of the protein tubulin
- Heterodimers of alpha and beta tubulin arranged into 13 protofilaments
- Asymmetry of protofilaments provides structure with polarity
- Primary functions include support, cell organization, and intracellular transport
- Forms mitotic spindles, centrioles, cilia, flagella



From *Karp's Cell and Molecular Biology* Figure 9.3d

Flagella

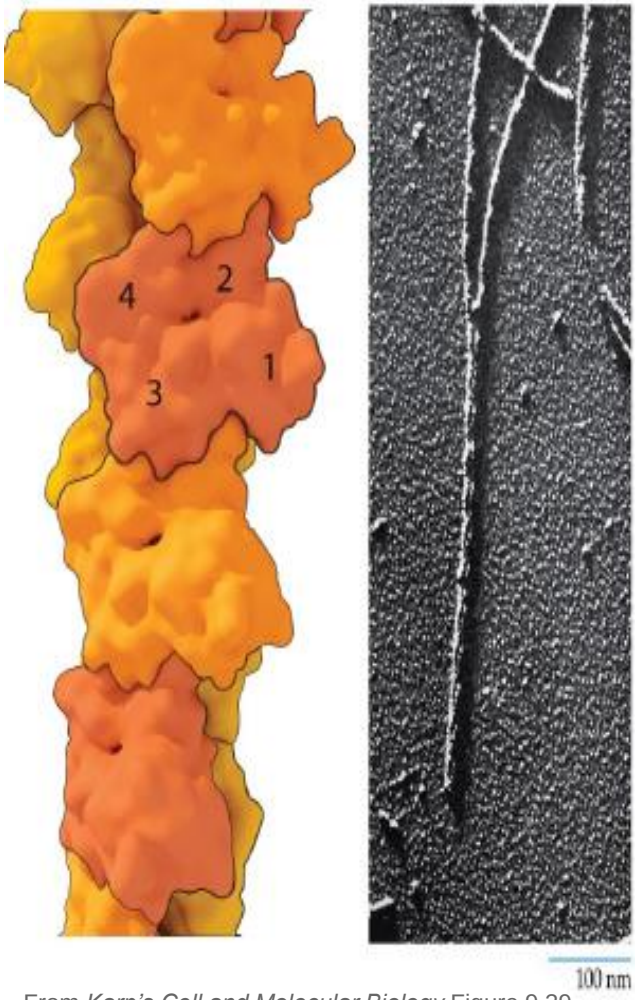


https://blogndrian106.blogspot.com/2022/11/cytoskeleton-cilia-and-flagella.html

- Organelles that project from the surface of cells
- Covered by a membrane that is continuous with the cell membrane
- Contains array of microtubules and microtubule associated proteins
  - Nine peripheral doublets
  - Two central microtubules
- Built by the addition of tubulin dimers to distal end of flagellum (+ end)
- Proximal end is anchored into cytoplasm via the basal body

Microfilaments

- Smallest of the three main components of the cytoskeleton
- Assembled from monomers of the protein actin, interacting in a single fiber
- Structure has polarity
- Primary functions include support and intracellular transport
- Also involved in dynamic alterations to cell shape
    - Cell motility
    - Cytokinesis



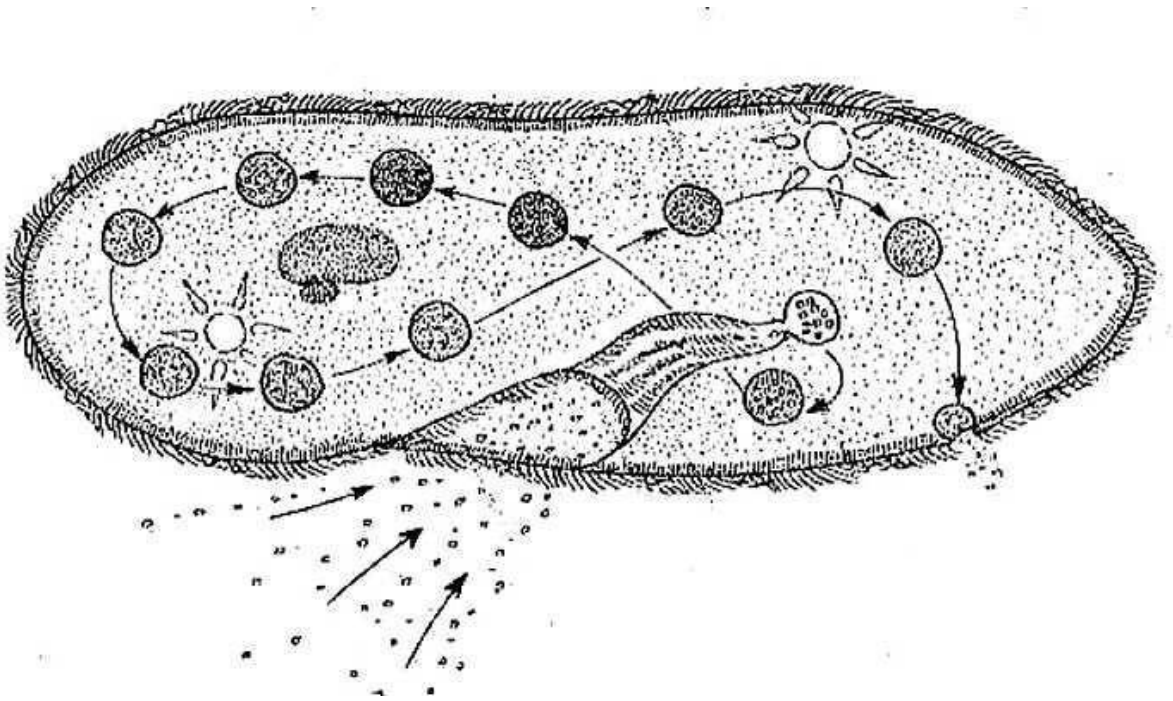
From *Karp's Cell and Molecular Biology* Figure 9.39

Phagocytosis

- “Cell eating”
- Performed by cells specialized for the uptake of particulate matter from their environment

Stages of phagocytosis:

1. Ingestion – food enters through oral groove, food vacuole forms
2. Acidification – to decrease the pH of the vacuole
3. Digestion – occurs when digestive lysosome fuses with vacuole
4. Egestion – waste released through anal pore



Lab 3: Examination of the Cytoskeleton

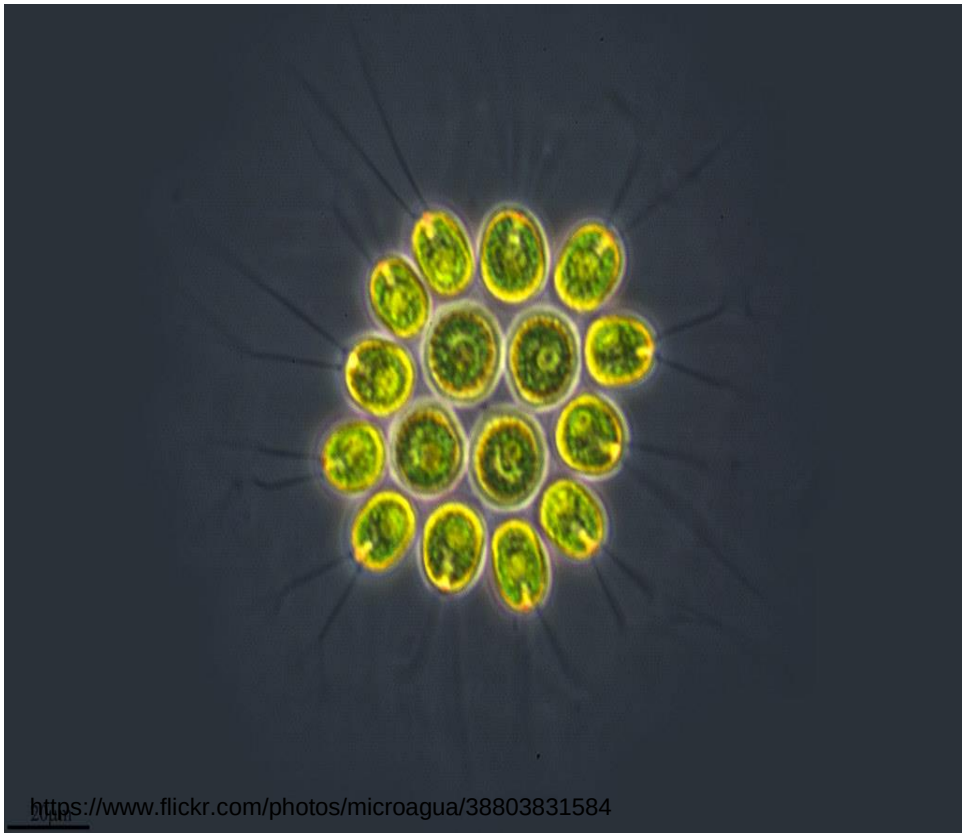
Effects of inhibitors on the regeneration of flagella

- Deflagellation and drug treatment
- Phase contrast microscopy and measurement of flagella length
- Data analysis

Lab 3: Examination of the Cytoskeleton

Effects of inhibitors on the regeneration of flagella

- *Gonium* sp. used as model
  - Biflagellate green algae
- Deflagellation performed through mechanical shearing in a blender
- Compare natural rate of flagellar regeneration to conditions with:
  - Cycloheximide
  - Colchicine
  - Cytochalasin B
- Cells treated for 120 minutes, with measurements taken every 20 minutes



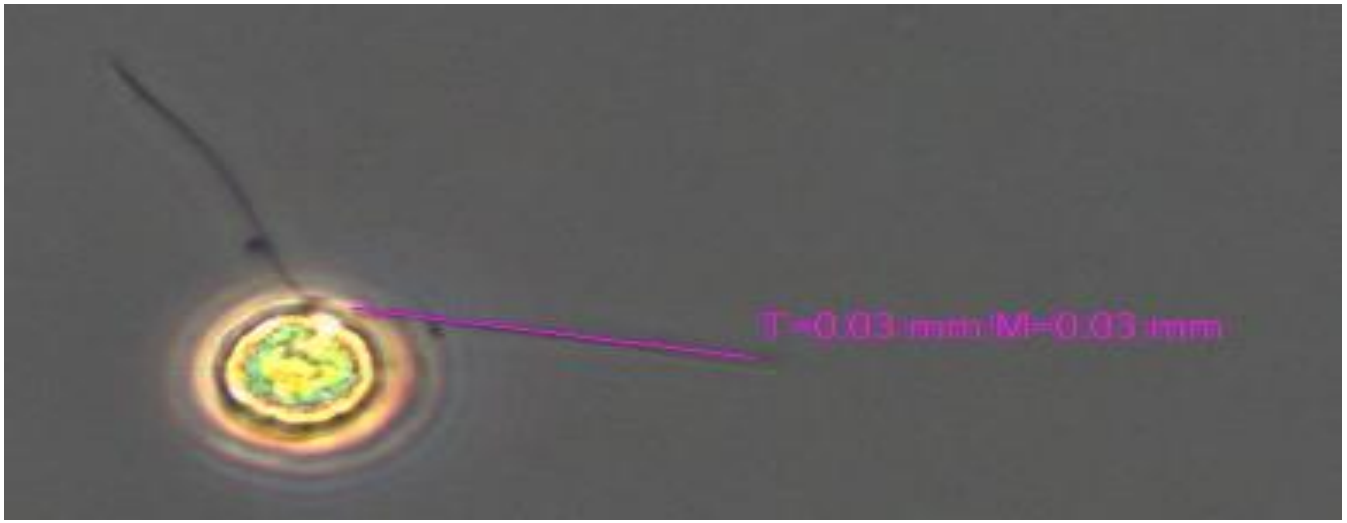
Lab 3: Examination of the Cytoskeleton

Effects of inhibitors on the regeneration of flagella

- Five experimental conditions to be observed:
  - Nondeflagellated
  - Medium (natural regeneration)
  - Cycloheximide
  - Colchicine
  - Cytochalasin B

- 3 drops of culture mixed with 2 drops of glutaraldehyde for fixation before viewing

- Measurements taken using phase contrast microscopy & Infinity Analyze software



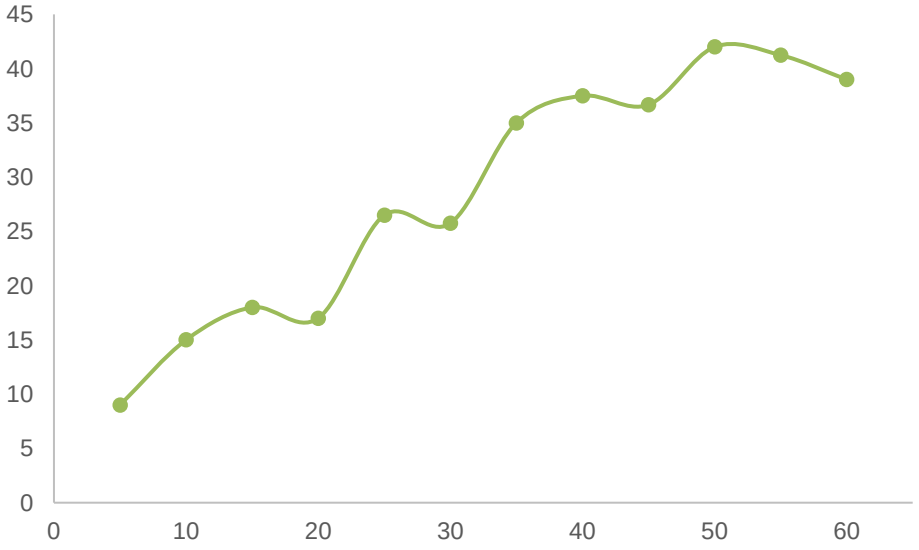
Lab 3: Data Analysis

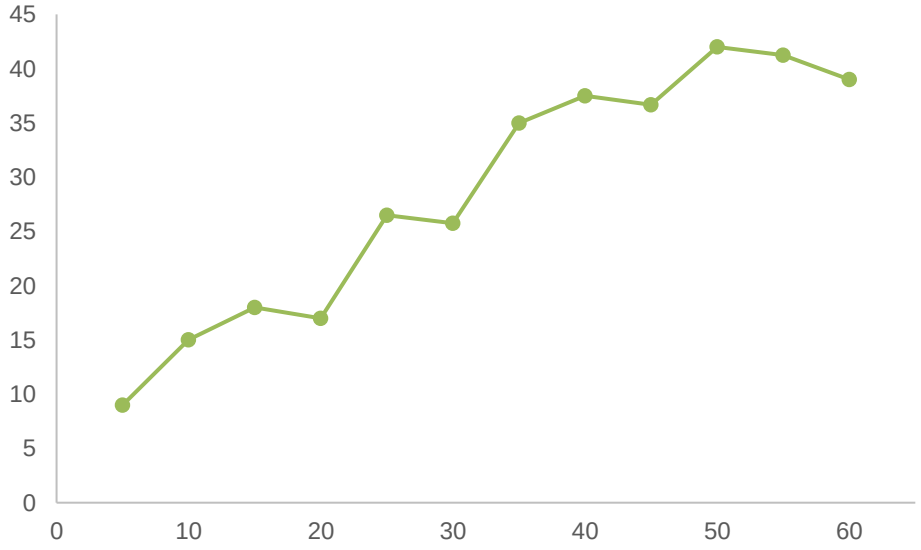
Effects of inhibitors on the regeneration of flagella

- Calculate the average and standard deviation for the lengths from each treatment and time

- Prepare a single graph comparing the length of the flagella (μm) over time
    - Include all five treatments on a single graph with a descriptive legend

- Use curved connecting lines and custom error bars





Lab 3: Examination of the Cytoskeleton

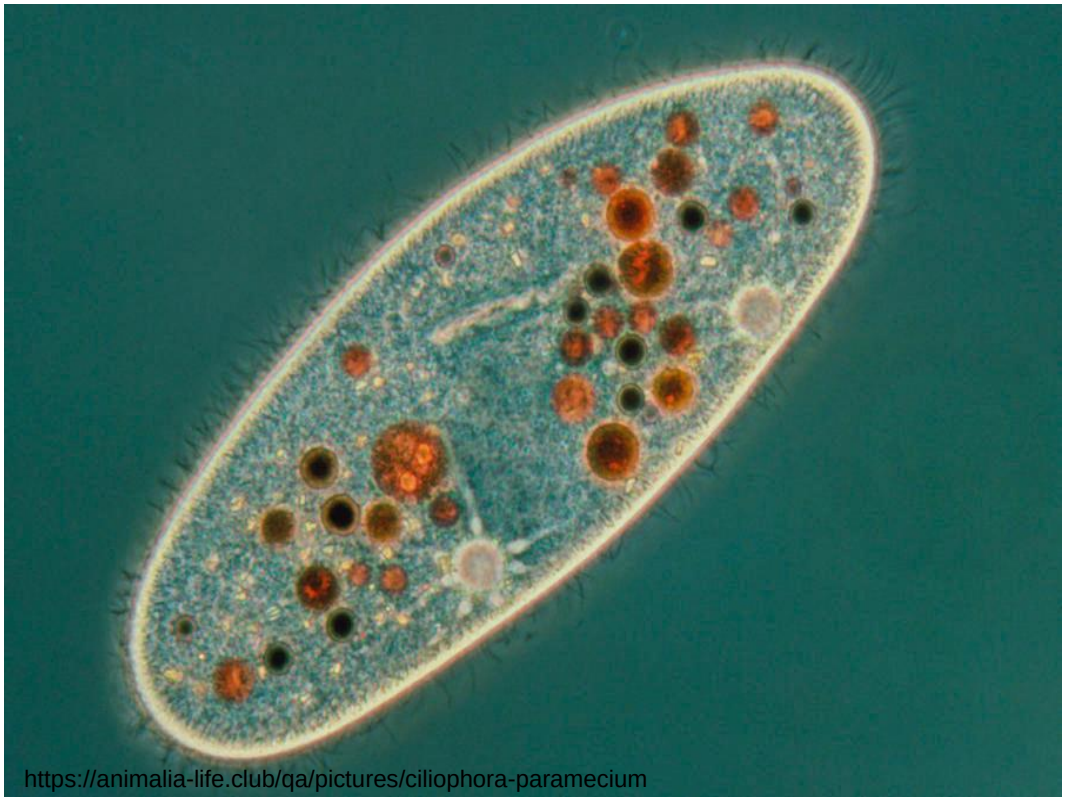
Phagocytosis assay

- Sample preparation
- Phagocytosis assay
- Data analysis

Lab 3: Examination of the Cytoskeleton

Phagocytosis assay

- *Paramecium* used as model
  - Unicellular ciliate eukaryote
- 0.5 mL of concentrated culture mixed with 50 µL of carmine red
  - Used as “food” to count food vacuoles
- The stained culture was then immobilized using 12% polyvinyl alcohol (PVA)
- Examine using bright-field microscope



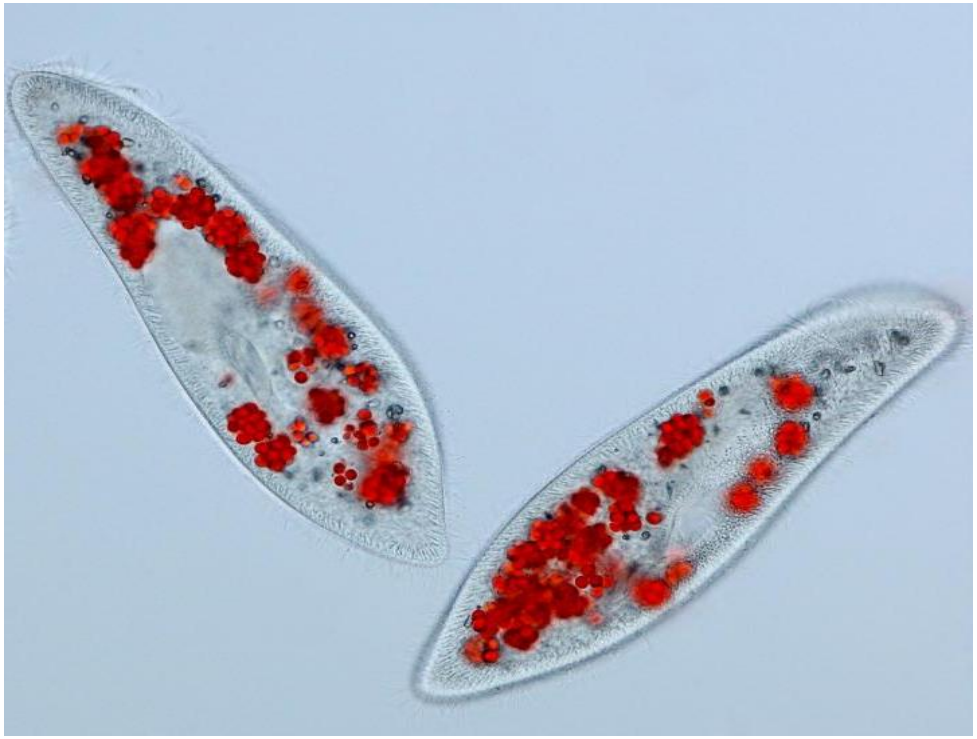
https://animalia-life.club/qa/pictures/ciliophora-paramecium

Lab 3: Examination of the Cytoskeleton

Phagocytosis assay

- Assay run for 60 minutes, with measurements taken every 5 minutes

- Experimental conditions:
  - Untreated
  - Colchicine
  - Cytochalasin B



Lab 3: Data Analysis

Effects of inhibitors on the regeneration of flagella

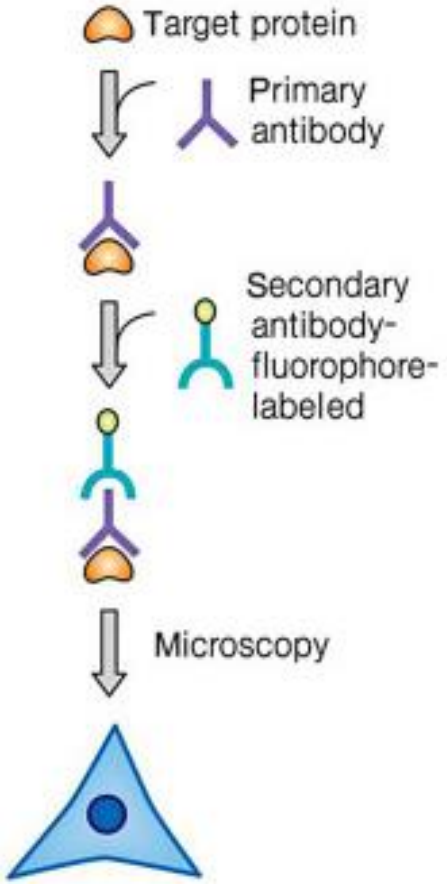
- Calculate the average and standard deviation for the number of food vacuoles obtained for each treatment and time point

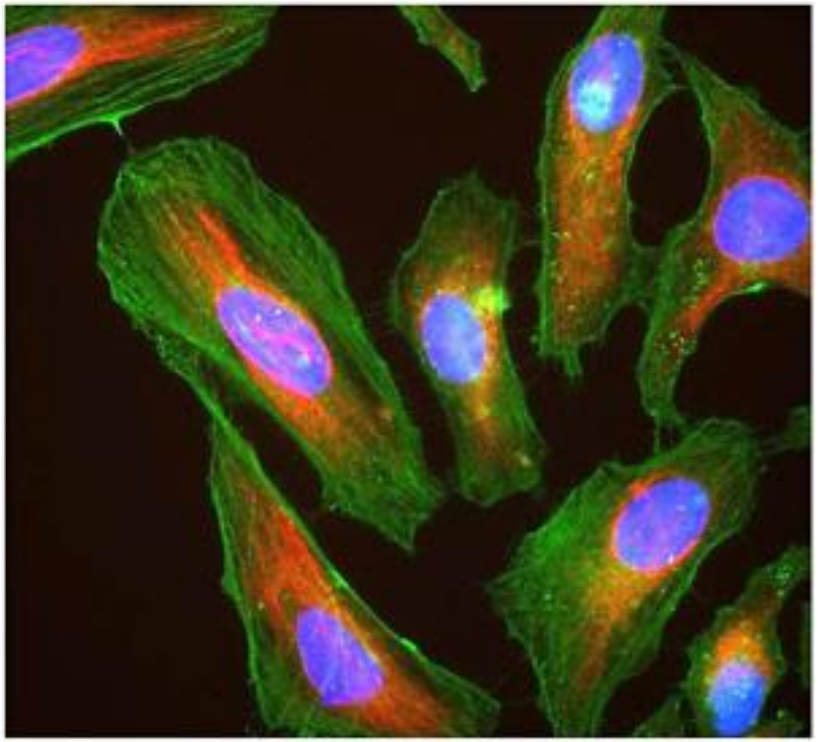
- Prepare a single graph comparing the number of food vacuoles over time
    - Include all three treatments on a single graph with a descriptive legend

- Use curved connecting lines and custom error bars

Lab 3: Examination of the Cytoskeleton

Demonstration of fluorescence microscopy



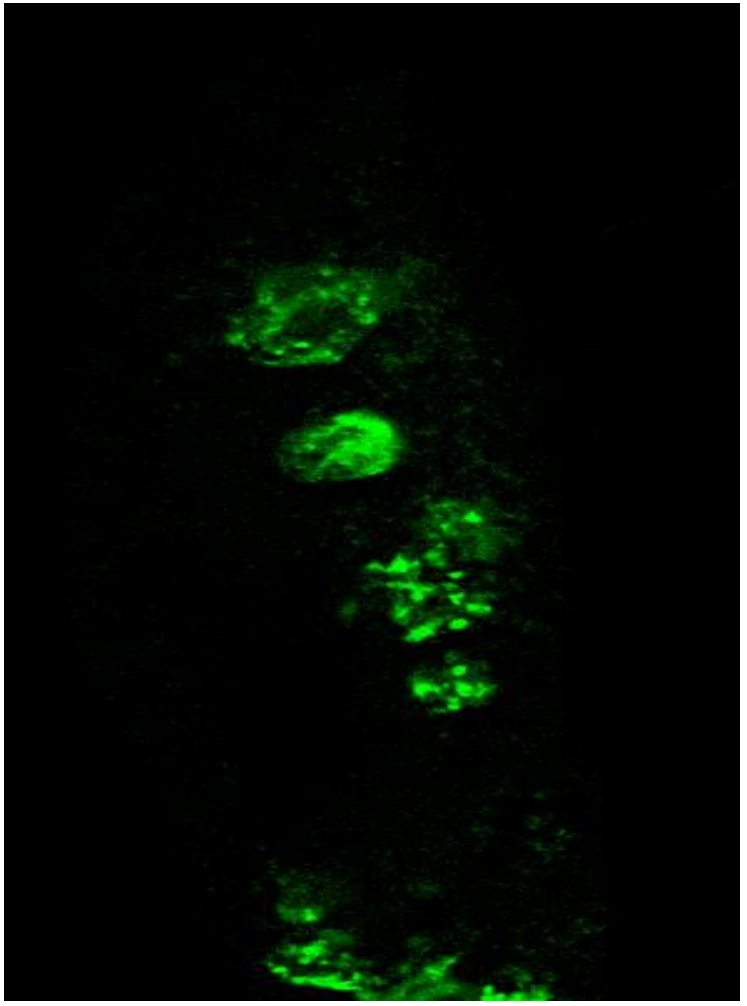


https://www.sciencedirect.com/topics/nursing-and-health-professions/immunofluorescence-test

Lab 3: Examination of the Cytoskeleton

Demonstration of fluorescence microscopy

- Staining of *Paramecium* with actin-specific fluorescently-labelled antibodies
- Our stain competes with phalloidin for binding to actin
    - Phalloidin binds to actin and stabilizes microfilaments
- Will show staining surrounding food vacuoles, where actin will have accumulated



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Lab 3: Examination of the Cytoskeleton

ANY QUESTIONS?

Lab 3 Assignment

- Formal “short” report
- Assignment guidelines and data are posted on Nexus
- Worth 10% of final grade
- Due by 11:59 PM on November 20th