Lab 2 Session 3 Microscopy and Cell Motility (Live Cells & Bacterial Motion)

Date: 10 Feb 2026
Lab Partner: Nathan Unhorn
Recorder: Ahilan Kumaresan

NOTICE: NO More E. coli or Mutant. Only Onion cells available. Session pivoted to: SFU pond water, yeast, and HT1080 stained slides.

IMPORTANT NOTE - SESSION 3 CHANGES
Original plan: Investigate E. coli bacterial motion
Actual session: E. coli cells were NOT available

Instead, we:
1. Collected water from SFU pond to find natural microorganisms
2. Imaged yeast cells (fresh batch, different dilutions)
3. Imaged pre-stained HT1080 malignant cells

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I. GOALS (Revised for Session 3)

Original goals (E. coli-based):

1. Prepare E. coli sample - NOT POSSIBLE (no E. coli available)

Revised goals for this session:

1. Collect natural water sample from SFU pond
2. Prepare samples and search for microorganisms
3. Image and measure any cells/organisms found
4. Image fresh yeast at different dilutions
5. Image pre-stained HT1080 malignant cells
6. Identify unknown organisms if possible
7. Plan for next session: find MOVING organisms

II. APPARATUS

Standard Equipment: Refer to Session 1, Section II

Item	Description
Microscope	Olympus BX51 upright bright-field
Camera	FLIR BlackFly U3-13Y3M (1440 x 1080 px)
Objective used	100x oil immersion (all imaging today)
Immersion oil	n = 1.518
Stage micrometer	1mm/100 div (1 div = 0.01 mm = 10 um)
Software	Vision Assistant, ImageJ

Samples for Session 3:

Sample	Source	Notes
SFU Pond water	Collected by technician Selvesta from SFU pond	Collected from pond bottom (warmer)
S. cerevisiae (yeast)	Fresh batch, baker's yeast	Different dilutions tested
HT1080 cells	Pre-prepared stained slide	Blue-stained, fixed cells

Materials for pond water collection:

Item	Description
Large glass jar	Inverted with air trapped inside
Collection	Placed at pond bottom, then inverted to

III. VARIABLES

Variable Type	Variable	Description
Independent	Sample type	Pond water, yeast, HT1080
Independent	Chamber type	With spacer vs without spacer
Dependent	Cell size (um)	Measured from images
Dependent	Cell morphology	Shape, internal structures
Control	Temperature	Room temperature (~22 C)
Control	Objective	100x oil immersion

IV. CALIBRATION (100x Objective)

(Refer: MM-LabScript Section 3.1, p.6-7)

Calibration slide specifications:
1 division = 0.01 mm = 10 um

Measurement in ImageJ:
Number of lines measured: 8
Total physical distance: 8 x 10 um = 80 um
Pixel count: 1170 px

Calculation:
Pixel size = 80 um / 1170 px = 0.0684 um/px

(7)

Comparison to previous sessions:

Session	Calibration (nm/px)	Objective
Session 1	68.45	100x
Session 2	345	100x
Session 3 (today)	68.4	100x

NOTE: The Session 2 calibration (345 nm/px) appears to be an ERROR.

Files:
calibrate0000.tif (path: Data/10-Feb/calibrate0000.tif)
calibrate0001.tif (path: Data/10-Feb/calibrate0001.tif)

CONCLUSION: Calibration verified at 68.4 nm/px for 100x objective.

The Session 2 error may have been caused by:
- Wrong number of divisions counted
- Calculation error
- Possibly using wrong objective setting???

V. SFU POND WATER COLLECTION

Collection performed by: Technician Selvesta (with our request)
Location: SFU campus pond
Date of collection: 10 Feb 2026

Collection method:
1. Large glass jar was inverted (trapping air inside)
2. Jar placed at BOTTOM of pond
Reason: Bottom is warmer, more likely to have living
3. Jar inverted underwater to collect water and sediment

Initial observations of collected water:
Appearance: Largely clear
Impurities: Large visible particles/debris present
Color: Light Brown particles at the bottom
Smell: None

CONCLUSION: Successfully collected pond water from SFU campus.
Water appears clear with some debris. Will incubate at room temperature to encourage organism growth for next session.

VI. POND WATER SAMPLE PREPARATION AND

(Refer: Protocol: Making Sample Chambers)

6.1 Sample Preparation - Comparison of Chamber

We tested two chamber types:

Chamber Type 1: Thin chamber WITH Parafilm spacer
Observation: Less activity visible
Cell visibility: Just one large unknown obejct

Chamber Type 2: Thin chamber WITHOUT spacer
Observation: Much MORE activity and cells visible
Cell visibility: Better

Best method: Chamber WITHOUT spacer (thin wet mount)
Reason: Without spacer, sample is thinner and organisms are

6.2 General Observations of Pond Water Sample

Objective: 100x oil immersion

What we saw:
- Various particles and debris
- At least two distinct cell types (Unknown 1 and Unknown 2)

Search for motile organisms:

Did we find swimming/moving cells? NO

Plan: Let water incubate, check again next session

CONCLUSION: Pond water contains cells/organisms but no motile (moving) cells were found today. Will check again

VII. UNKNOWN CELLS FROM POND WATER

7.1 Unknown Cell 1 - Possible Arcella or Air Bubble/Spore

File: unknown.tif (path: Data/10-Feb/unknown.tif)
Objective: 100x

Measurements in ImageJ:

Measurement	Value (px)	Value (um)
Minor axis	801.29	54.8
Major axis	841.76	57.6

Size calculation (using calibration 0.0684 um/px):

Minor axis: 801.29 x 0.0684 = 54.8 um
Major axis: 841.76 x 0.0684 = 57.6 um
Shape: Roughly circular/oval (~55-58 um diameter)

Observations:
- Has a circle inside, but it is OFF-CENTER (not in middle)
- When glass slide is pressed slightly, shape CHANGES

Possible identification:

Candidate	Evidence For	Evidence Against
Arcella (testate amoeba)	Roughly correct size (~50-100 um)	Arcella has CENTERED aperture, this is off-center

Best guess: Uncertain - possibly air bubble or spore. The off-center internal structure and shape-changing behavior

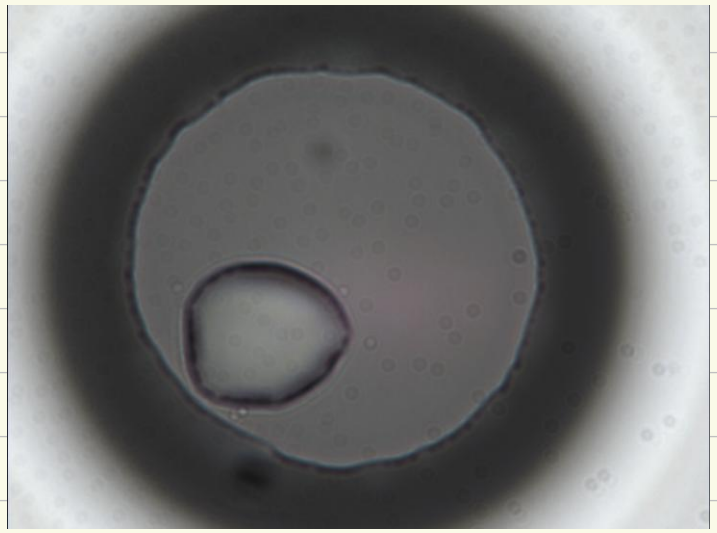


Image 1: Unknown cell 1 from SFU pond water. Size ~55-58 um. Possible air bubble or spore. Note off-center internal circle.

CONCLUSION: Unknown 1 is approximately 55-58 um in diameter. Most likely an air bubble or spore rather than Arcella due to off-center internal structure. Changes shape under pressure.

7.2 Unknown Cell 2 - Possible Pandorina

File: unknown2.tif (path: Data/10-Feb/unknown2.tif)
Objective: 100x

Measurements in ImageJ:
Minor axis endpoints: (596, 440) to (729, 592)
Minor axis length: 205.006 px
Major axis endpoints: (565, 632) to (753, 425)
Major axis length: 281.192 px

Size calculation (using calibration 0.0684 um/px):
Minor axis: 205 x 0.0684 = 14.0 um
Major axis: 281 x 0.0684 = 19.2 um
Shape: Oval/elliptical (~14 x 19 um)

Possible identification:

Candidate	Typical size	Match?
Pandorina (colonial green alga)	~20-50 um colony	Size is on smaller end but possible

Pandorina characteristics:
- Colonial green alga (8-16 cells in a colony)
- Cells arranged in a sphere/oval
- Each cell has 2 flagella (colony can swim!)
- Found in freshwater ponds

Note: If this IS Pandorina, it should be capable of
We may see it move in future sessions after

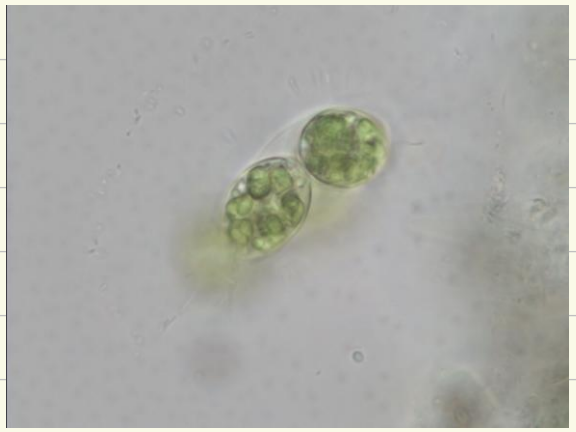


Image 2: Unknown cell 2 from SFV pond water. Size ~14 x 19 um. Possibly Pandorina (colonial green alga).

CONCLUSION: Unknown 2 is approximately 14 x 19 um. Possibly Pandorina based on size and shape. If correct, may

7.3 Summary of Unknown Cells

Cell	Size (um)	Possible ID	Motile?	File
Unknown 1	~55-58 (diameter)	Air bubble/spore	No	unknown.tif
Unknown 2	~14 x 19	Pandorina	Potentially (if Pandorina)	unknown2.tif

CONCLUSION: Found two distinct objects in pond water. Neither showed movement during this session.

VIII. YEAST IMAGING (Fresh Batch)

(Refer: MM-LabScript Section 3.3, p.9)

Time: 2:36 PM

8.1 Yeast Sample Preparation

Fresh batch of yeast prepared.
Tested different DILUTIONS to find optimal density.

Dilutions tested:

- 1:10 dilution (10x)
- 1:100 dilution (100x)
- 1:200 dilution (200x)

8.2 Yeast Imaging Results

Files collected:

Filename	Dilution	Type	Path
yeast-dilution-10x.tif	1:10	Image	Data/10-Feb/yeast-10x.tif
yeast-dilution-10x.avi	1:10	Video	Data/10-Feb/yeast-10x.avi
yeast-dilution-100x.avi	1:100	Video	Data/10-Feb/yeast-100x.avi
yeast-dilution-200x.avi	1:200	Video	Data/10-Feb/yeast-200x.avi

Note: Original filenames use '10x', '100x', '200x' which refers
to DILUTION, not objective magnification.

8.3 Yeast Motion Observations

Motion observed in videos:

- Cells VIBRATE (Brownian motion visible)
- NO DIRECTED motion observed
- Yeast cells do not actively swim

This is expected: Yeast (S. cerevisiae) is non-motile.
The vibration is thermal Brownian motion, not self-

8.4 Future Experiment Idea: Sugar Gradient

Idea: Place sugar on one side of the chamber to create a
concentration gradient. Yeast may show chemotaxis

This could be a potential project for Sessions 4-6:
- Measure yeast 'diffusion' rate in response to sugar
- Compare to Brownian motion of beads
- Investigate if yeast shows any directed motion

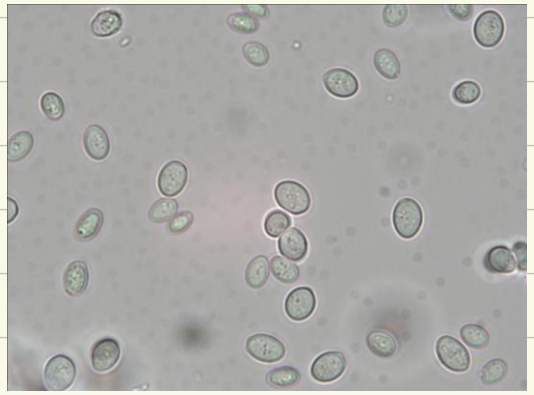


Image 3: Yeast cells (S. cerevisiae) at 1:10 dilution, 100x.

CONCLUSION: Yeast cells show Brownian vibration but no directed
motion. Future experiment: use sugar gradient to

IX. HT1080 MALIGNANT CELLS

(Refer: MM-LabScript Section 3.3, p.9)

9.1 HT1080 Slide Information

Sample type: Pre-prepared fixed slide
Staining: Blue-stained (pre-stained)
Cell type: HT1080 - malignant human fibrosarcoma

Background from labscript:
HT1080 cells are epithelial cells derived from fibrosarcoma
cells, which are malignant tumor cells from connective tissue.
Because they are tumor cells, they proliferate easily

9.2 HT1080 Observations

Objective: 100x

Files:
ht1080.tif (path: Data/10-Feb/ht1080.tif)
ht1080-2.tif (path: Data/10-Feb/ht1080-2.tif)

Observations:
- Cells appear with web-like/irregular structures
- NO uniform shape observed
- Difficult to define clear cell boundaries
- Blue staining visible

Size measurement: NOT MEASURED - cells did not have a uniform
shape to measure. The web-like structures made it

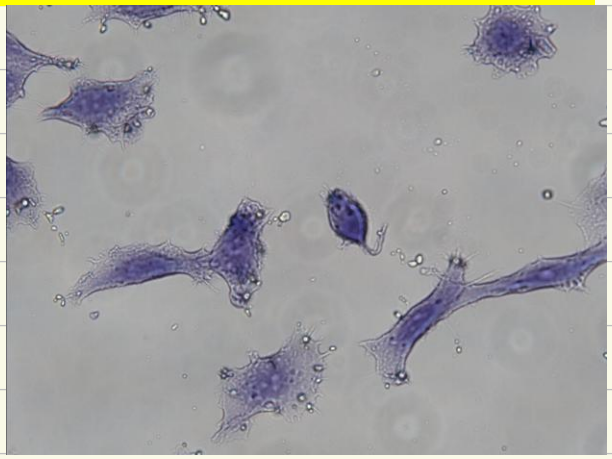


Image 4: HT1080 malignant cells (stained blue) at 100x. Note the irregular, web-like structure.

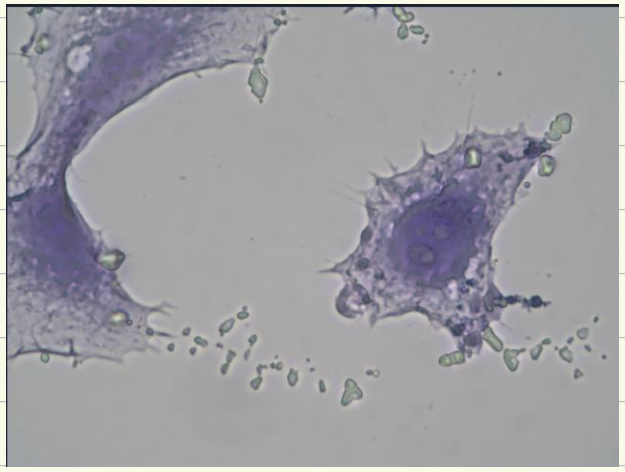


Image 5: HT1080 malignant cells (second image) at 100x.

CONCLUSION: HT1080 cells observed but size not measured due to irregular, web-like morphology. Cells do not have uniform shape like yeast or bacteria.

X. CONCLUSIONS

10.1 Summary of Session 3 Results

What we accomplished:

Task	Status
Calibration (100x)	DONE - 68.4 nm/px
SFU pond water collection	DONE - collected from pond bottom
Pond water imaging	DONE - found 2 cell types
Unknown cell 1	Measured: ~55-58 um (bubble/spore?)
Unknown cell 2	Measured: ~14 x 19 um (Pandorina?)
Yeast imaging	DONE - 3 dilutions, videos
HT1080 imaging	DONE - 2 images
Find moving cells	NOT FOUND - defer to next session

10.2 Key Findings

1. Calibration: 68.4 nm/px (matches Session 1, Session 2

Session 2 calibration (345 nm/px) is WRONG!
Correct value: 68.4 nm/px (confirmed Sessions 1 and

2. Pond water: Contains cells/debris but no motile organisms yet
3. Unknown cells:
   - Cell 1: ~55-58 um, possibly air bubble or spore
   - Cell 2: ~14 x 19 um, possibly Pandorina (colonial alga)

10.3 What We Did NOT Accomplish (Deferred)

- E. coli imaging (not available)
- Finding and tracking MOVING cells
- Quantitative motion analysis (MSD, D_eff)

These are goals for Session 4 and beyond.

XI. PLAN FOR SESSION 4 AND BEYOND

11.1 Immediate Plan for Session 4

1. Check incubated pond water for organism growth
   - Water has been at room temp (~22 C) since collection
   - Expect more organisms may have grown
2. FIND MOVING CELLS
   - This is our primary goal
   - Once found, perform quantitative tracking

11.2 Project Plan Options (for Sessions 4-6)

Since E. coli is NOT available, we propose the

PROJECT 1: Onion Cell Intracellular Streaming

(Refer: MM-LabScript Section 3.5, p.10)

Goal: Observe and measure cytoplasmic streaming in onion cells

Method: Peel thin membrane from onion, mount on slide

PROJECT 2: Bead Diffusion - Size, Concentration,

(Refer: MM-LabScript Sections 3.2)

Goal: Investigate how D changes with experimental parameters
Variables to test:
- Bead SIZE: 1 um vs 5 um vs other sizes
- Bead CONCENTRATION: dilute vs concentrated
- VISCOSITY: water vs glycerin mixtures

PROJECT 3 (If time): Motivated Yeast Experiment
Goal: Test if yeast shows chemotaxis toward sugar
Method: Create sugar gradient in chamber
Analysis: Track yeast positions, look for drift

PROJECT 4 (If time): Campus Sample Collection
Goal: Collect samples from various surfaces around campus
Method: Swab surfaces, culture in water, image

11.3 Pond Water Incubation

Current status: Pond water stored in lab at ~22 C
Expected outcome: Warmer temperature promotes organism growth
Check next session for:
- Increased cell density
- New organism types

XII. POST-LAB REFLECTIONS

What worked well:

1. Pond water collection was successful
2. Found interesting unknown cells to investigate
3. Calibration matches Session 1 (resolved

Issues encountered:

Issue	Cause	Solution
No E. coli available	Lab supply issue	Pivot to pond water project
No moving cells found	Pond water may need incubation	Let water incubate, check next session
HT1080 size not measurable	Irregular cell morphology	Accept as observation, focus on other cells

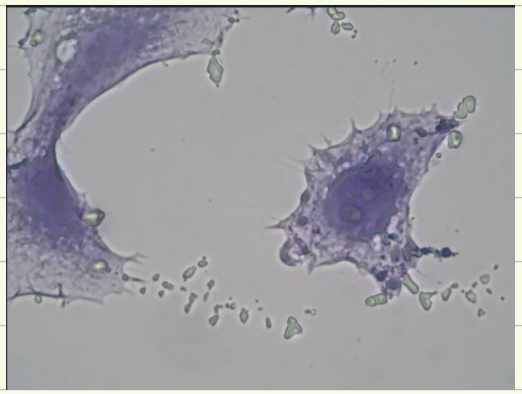
Questions for TA/Prof:

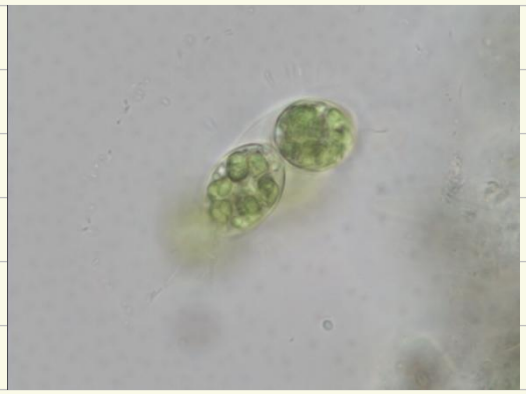
1. Can we confirm identification of Unknown 1 and Unknown 2?
2. Is the Session 2 calibration (345 nm/px) definitely

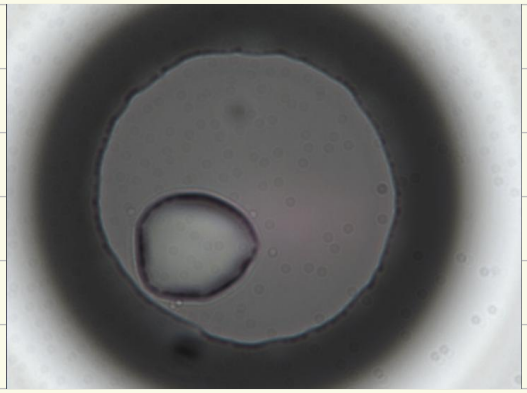
DATA FILES SUMMARY

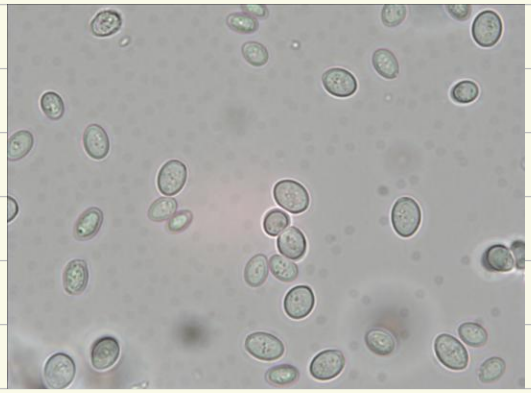
All files in: Data/10-Feb/

Cool Images Gallery, We collected and I am proud of









Session 3 completed: 10 Feb 2026

Lab [2] Session [4] M&M: Short Project Exploration

Date: 12 Feb 2026
Lab Partner: Nathan Unhorn
Recorder: Ahilan Kumaresan

NOTICE: E. coli HCB1274 culture is still NOT available.
This session focuses on exploring 2-3 candidate

Repository: github.com/Ahilan-Bucket/phys332W-sfu/tree/main/Lab2-Microscopy-and-Motility

1. GOALS

1. Verify calibration (should match ~68 nm/px)
2. Check incubated pond water for motile organisms (3 days)
3. If motile organisms found: capture video, track with MTrack2
4. Explore onion cell streaming as candidate project
5. Explore bead diffusion under corrected calibration

2. APPARATUS

Standard Equipment (same as Sessions 1-3):

Refer to Session 1, Section II (pg 2 of lab notebook)

Additional Items for Session 4:

Item	Purpose
Incubated pond water	Motile organism search
Onion	Intracellular streaming
Tweezers	Peel onion membrane
1 um beads	Diffusion comparison
5 um beads	Diffusion comparison
Glass slides	Sample mounting
Coverslips (#1)	22x22 mm
Parafilm spacers	~100 um chambers
Nail polish	Seal chambers

3. VARIABLES

Type	Variable	Description
Independent	Sample type	Pond water / onion / beads
Independent	Incubation time	3 days for pond water
Independent	Chamber type	With/without spacer
Dependent	Organism density	Cells per FOV
Dependent	Cell size (um)	From images
Dependent	Motion type	Swimming / streaming / Brownian
Dependent	Swimming velocity	um/s
Dependent	D_eff	Effective diffusion coeff.
Dependent	MSD(tau)	Mean-squared displacement
Dependent	alpha (MSD exp.)	MSD ~ t^alpha
Control	Temperature	~22 C (room temp)
Control	Objective	100x oil immersion
Control	Pixel size	68.4 nm/px (calibrated)
Control	Frame rate	1 fps

4. REFERENCES

Primary Lab Documents:

1. MM-LabScript-microscopy.pdf (Sec 3.3-3.5, pp. 9-11)
2. CellMotility-LabScript.pdf (cell motion background)
3. Protocol: Microscope Setup -- Olympus BX51
4. Protocol: Making Sample Chambers
5. Protocol: Tracking Particles (MTrack2)
6. Protocol: Acquiring Movies with Vision Assistant

Scientific References:

7. UCB Advanced Lab BMC -- intracellular movement

Previous Lab Data:

10. Session 1-2 Diffusion Analysis:
Diffusion_Analysis.ipynb

Textbooks:

12. Hughes & Hase, Measurements and Uncertainties (Ch

5. MICROSCOPE SETUP VERIFICATION

(Must do every session)
Ref: Protocol: Microscope Setup -- OLYMPUS BX51
Time: ~1:30 PM

5.1 Kohler Illumination

Step	
Lamp on, 5 min warm-up	Yes
Blank slide, focus at 10x	Yes
Field diaphragm edges sharp	Yes
Condenser centered	Yes
Field diaphragm to just outside FOV	Yes
Aperture diaphragm to ~70% NA	Yes

5.2 Calibration Verification (100x)

File: Data/12-Feb/calibration-100x-feb-12.tif

Stage micrometer measurement:

- Number of lines: 9
- Physical distance: 9 x 10 um = 90 um
- Pixel count: 1310 px
- Pixel size = 90 um / 1310 px = 68.7 nm/px

CONCLUSION: Calibration is 68.7 nm/px.
This MATCHES Sessions 1 (68.45) and 3 (68.4).

6. CALIBRATION ERROR HISTORY

6.1 Calibration Across All Sessions

Session	Date	Calibration	Status
Session 1	03 Feb 2026	68.45 nm/px	REFERENCE
Session 2	05 Feb 2026	345 nm/px	ERROR -- WRONG
Session 3	10 Feb 2026	68.4 nm/px	CONFIRMED
Session 4	12 Feb 2026	68.7 nm/px	CONFIRMED

IMPORTANT: Session 2 calibration (345 nm/px = 0.345 um/px) was WRONG.
Correct value: 68.4 nm/px = 0.0684 um/px.
All Session 2 D values inflated by ~25x (D scales as

Note: There appears to be an additional ~2x digital zoom from the
camera/screen software. Check FLIR BlackFly camera

Action today: Verified calibration matches ~68 nm/px.
File saved as: Data/12-Feb/calibration-100x-feb-12.tif

Note: Difficulty placing immersion oil when onion membrane
was on the slide. Recalibrated with calibration slide

6.2 Impact on Previous Results

Session 2 used 0.345 um/px. Correcting to 0.0684 um/px reduces all D by (0.345/0.0684)^2 = 25.4x.

Corrected values:

Bead (um)	D_MSD (Sess 2)	D_corrected	D_theory
1.0	12.88 um^2/s	0.507 um^2/s	0.4414
5.0	3.75 um^2/s	0.148 um^2/s	0.0883

CONCLUSION: 68.7 nm/px confirmed.
1 um corrected D within ~15% of Stokes-Einstein theory.
5 um corrected D still ~68% high (drift/noise).
Condenser shift is ~0.4%, within measurement uncertainty.

7. PROCEDURE A: POND WATER INCUBATION CHECK

Pond water collected 10 Feb 2026 from SFU campus pond

7.1 Visual Inspection (Before Microscope)

(For procedure refer to Session 3, Pond Water Section, Time: ~1:15 PM

We predicted 3-day incubation would show green tinge (algae

Collected a NEW swamp sample with improved technique:
Pipette near large masses/chunks. For jar: let water

Property	Session 3 (10 Feb)	Session 4 (today)
Water colour	Largely clear	Darker
Cloudiness	Can see through	Same
Visible debris	Large particles	Lesser
Green tinge?	Not noted	None
Smell	Not noted	Same
Sediment	Present	None

Summary: Incubated sample did not show increased organisms.
Fresh sample with improved technique showed more

7.2 Microscope Observation of Incubated Water

Time: ~1:20 PM

Sample: Wet mount WITHOUT spacer (best for pond

Objective: 40x overview, then 100x detail

Finding	Result
Organism density vs Session 3	Higher (incubation)
Unknown 1 (55-58 um, air bubble?)	Still present
Any NEW organism types	Yes – globular with villi
New MOTILE organisms found?	Minimal in new sample
Motile cells per FOV	~1-2 slow-moving

7.3 New Organism Identification

New Organism 3:

File: Data/12-Feb/unknown-3.avi | Objective: 100x

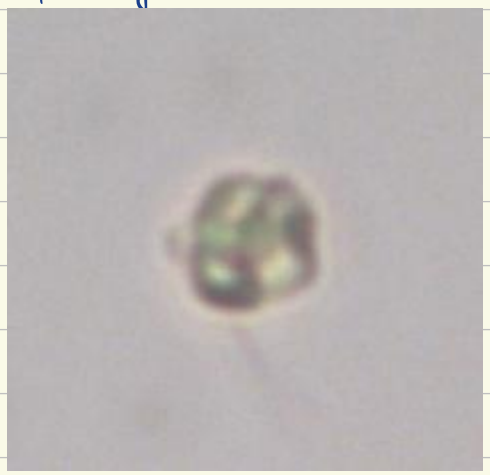


Image 1: Unknown organism 3 from incubated SFU pond water at 100x.

New Organism 4:

File: Data/12-Feb/unknown4.avi
Size: ~20 x ~15 um | Motion: Yes (minimal)
Shape: Globular, spherical
Possible ID: Unknown -- villi-like structures for propulsion

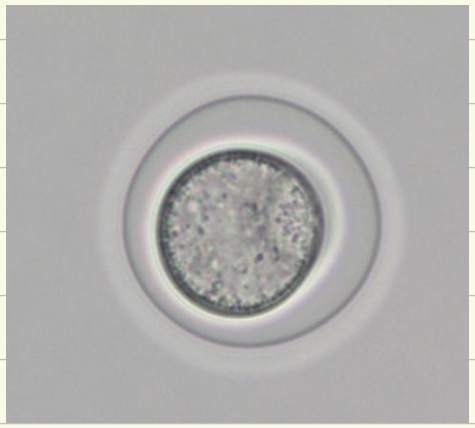


Image 2: Unknown organism 4 at 100x.

CONCLUSION: Our prediction was wrong – incubated water did not show more organisms. Fresh sample with improved technique

8. PROCEDURE B: ONION CELL EXPLORATION

(Priority #2 -- Estimated time: ~30 min)
Time: 2:04 PM

8.1 Background: Intracellular Streaming

Cytoplasmic streaming (cyclosis) = directed flow of cytoplasm
in plant cells. In onion epidermal cells:

- Organelles carried along actin filaments by myosin
- Streaming is directional (follows cell geometry -> nucleus)
- Literature speed: 1-10 um/s (slower than bacteria)
- ACTIVE transport, not passive diffusion

Key Q: How does intracellular transport differ from Brownian diffusion and free swimming?

8.2 Onion Preparation

Membrane quality: Good (some folding, usable regions
1. Cut onion in half
2. Separate inner layer (scale leaf)
3. Peel thin transparent membrane (epidermis)
4. Place membrane flat on glass slide
5. Add drop of water, lower coverslip at angle
6. Observe at 40x first, then 100x

8.3 Observations at 50x and 100x

Time: ~2:10 PM

50x observations:

- Cell walls visible: Yes
- Cell shape: Rectangular
- Estimated cell size: 110 x 904 px (~7.5 x 62 um)
- Streaming visible: Yes

100x observations (with oil):

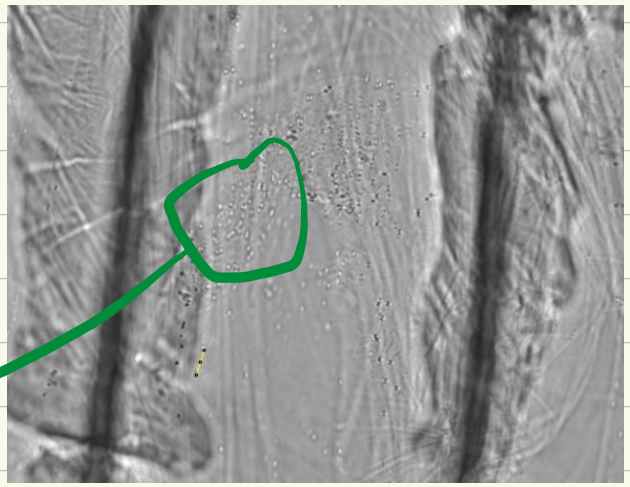


Image 4: Onion epidermis cells at 100x showing small granules of transport material.

Feature	Observation
Cell wall clarity	Very clear
Cytoplasm	Transparent -- may need stain
Organelles	None without stain
Streaming direction	Yes -- along the cell wall
Streaming speed	Fast (qualitative)
Granules/particles	Yes -- small, moving
Nucleus visible	Not yet (see Recording 2)

8.4 Video Capture of Cytoplasmic Streaming

Recorded at 1 fps (1 fps better for slow-moving

Recording 1 (Onion streaming):

Setting	Value
Filename	Data/12-Feb/onion-stream-01.avi
Objective	100x
Frame rate	1 fps
Frames	120
Duration	120 seconds
Notes	Nice but slightly out of focus

Recording 2:

Setting	Value
Filename	Data/12-Feb/onion2-stream.avi
Frame rate	1 fps
Frames	240
Duration	240 seconds
Notes	NUCLEUS VISIBLE! Material going towards it!

8.5 Quick Velocity Estimate

Pick visible granule, measure displacement over N

Granule 1: 44 px over 240 frames at 1 fps

$v = (44\ px)(0.0684\ \mu m/px)/(240\ s) = 0.0125\ \mu m/s$

Granule 2 (onion2-stream.avi): 50 px over 240 frames

$v = (50\ px)(0.0684\ \mu m/px)/(240\ s) = 0.0143\ \mu m/s$

Expected: 1-10 um/s (literature). Our values are lower --

CONCLUSION: Onion cell streaming clearly observed.
Estimated speed: 0.013-0.014 um/s (manual).
Viability as project: STRONG.

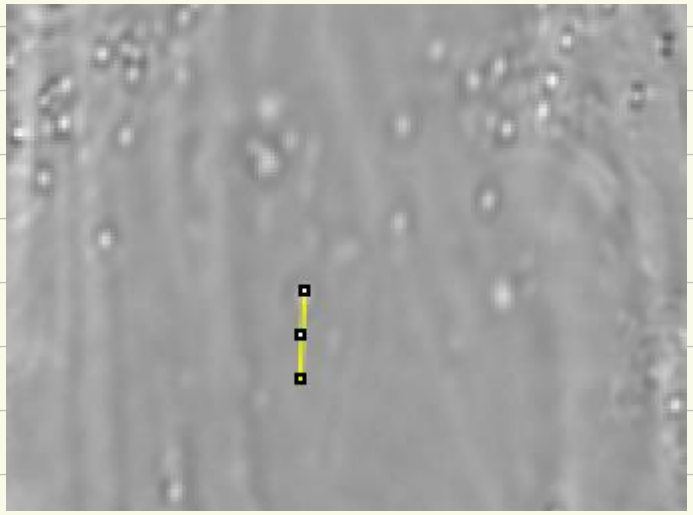


Image 5: Sample Image of Granule Velocity Calculations

8.6 Stained Onion Observations

Stained onion epidermis with Crystal Violet. Images improved dramatically -- nucleus and cell

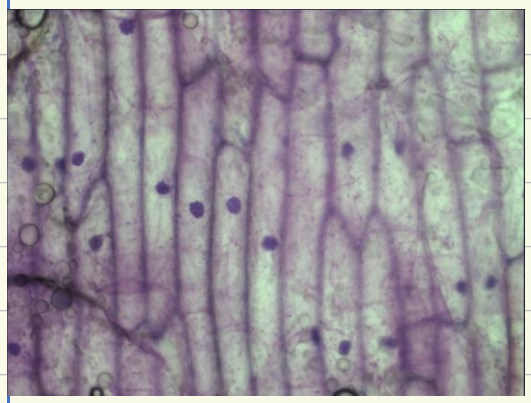
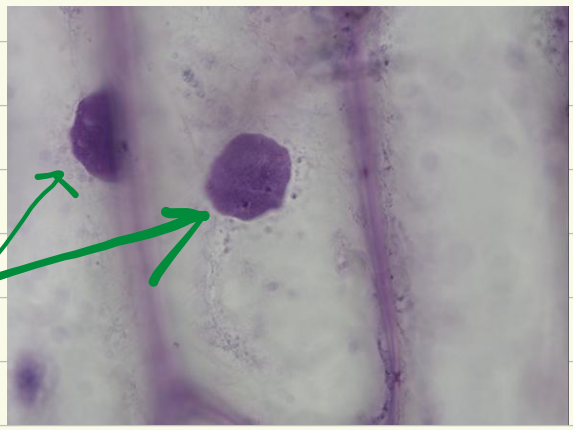


Image 6: Onion cells stained with Crystal Violet at 10x. Rod-like

Staining supports streaming data: more dye near nucleus,
consistent with active transport concentrating material there.
Independent visual evidence of directed transport!

Image 7: Onion cells stained with Crystal Violet at 50x, showing nucleus and cell wall.



Saved: onion-stained-10x.tif
and onion-stained-50x.tif

9. POST-LAB ANALYSIS

9.1 Qualitative Motion Comparison

System	Motion	Speed	Direction
1 um beads (S1)	Brownian	~0 net	Random
5 um beads (S2)	Brownian	~0 net	Random
Yeast (S3)	Brownian	~0 net	Random
Pond (today)	Minimal	Slow	Random
Onion streaming	Directed+random	0.013-0.023	Wall->nucleus

Progression from passive Brownian (beads, yeast) to directed
transport (onion) shows fundamental difference

9.2 Trajectory Analysis (Post-Lab Tracking)

ImageJ MTrack2 unreliable for onion granules (low contrast,
slow motion). Adapted our Session 1-2 diffusion notebook
(Diffusion_Analysis.ipynb) with new tracking front-end:

- track_onion_particles.py: background subtraction

Code: Analysis/track_onion_particles.py
Notebook: Analysis/Onion_Cell_Analysis.ipynb

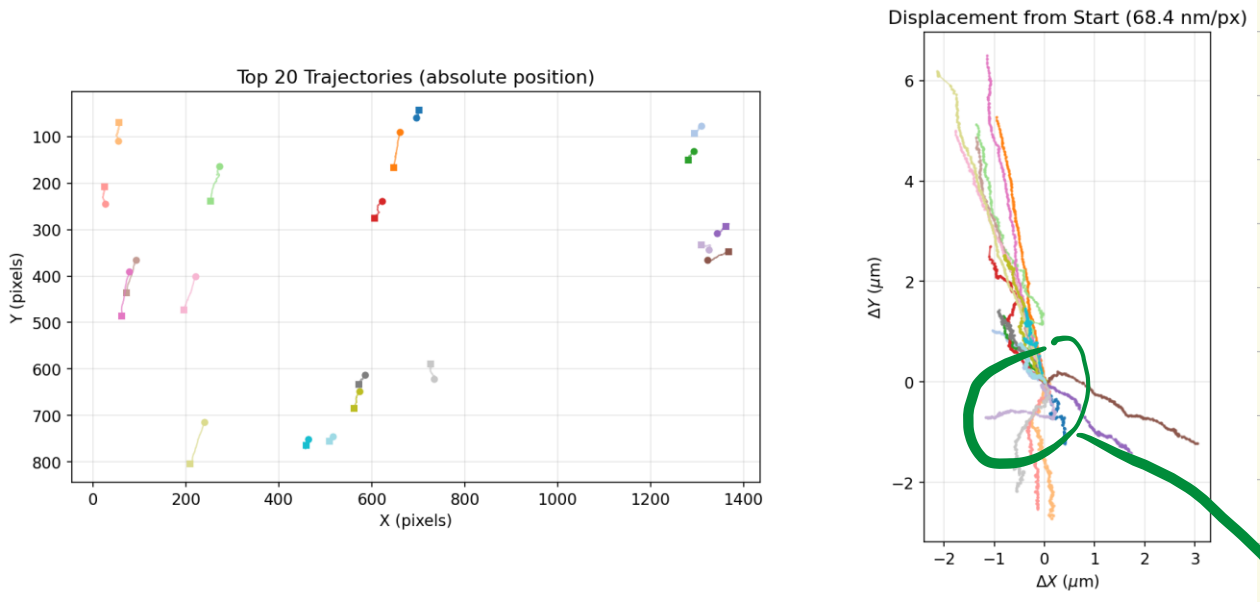


Figure 1: 2D trajectories of onion granules from onion2-streama.avi

Trajectories converge toward center (= nucleus). Consistent with Recording 2 observation: material going towards nucleus. This convergent pattern is cytoplasmic streaming.

Motion: Mixed (directed + random components)
Trajectory shape: Clearly a lot of them are going in one direction!!!

9.3 MSD Analysis

MSD(tau) = <[r(t+tau) - r(t)]^2>

Performed on top 20 longest track segments.

General power-law: MSD = K * tau^alpha

Pure Brownian diffusion: alpha = 1.0 (random walk)

Ballistic (fully directed): alpha = 2.0 (straight

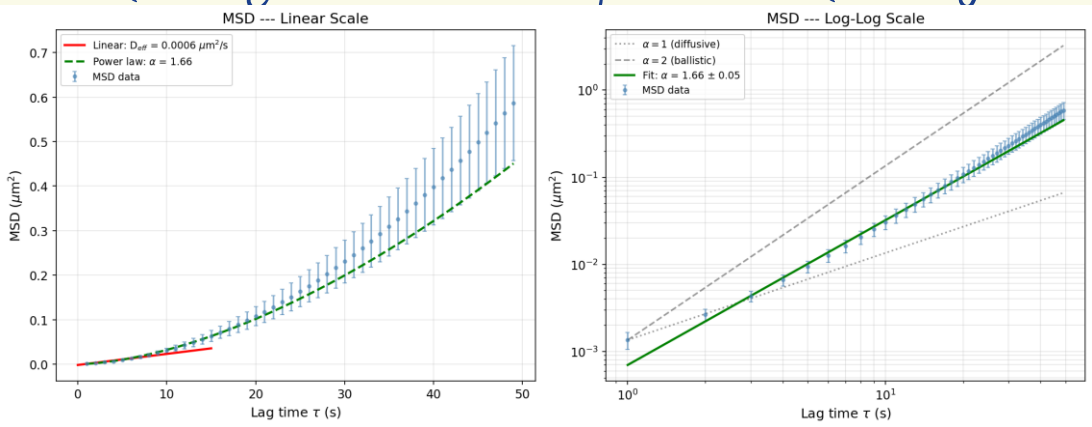


Figure 2: MSD log-log plot showing power-law fit with alpha = 1.66

Log-log MSD slope: alpha = 1.662 +/- 0.052
= SUPERDIFFUSIVE (between diffusion and ballistic).
Granules actively carried along actin filaments (alpha -> 2)
with random thermal fluctuations (alpha -> 1).

9.4 Velocity Estimates

Method	System	Velocity (um/s)	Notes
Manual	Granule 1	0.0125	44 px / 240 s
Manual	Granule 2	0.0143	50 px / 240 s
Automated	Top 20 mean	0.0228 +/- 0.0071	Tracking
Automated	Median	0.0177	Less outlier-sensitive
Literature	E. coli	~20	Reference (Becase we don't have the real one)

Automated tracking captures faster granules than manual.
Both consistent: streaming speed ~0.01-0.02 um/s, well

9.5 MSD Framework and Physical Constants

MSD(tau) = <[r(t+tau) - r(t)]^2>

2D Pure diffusion: MSD = 4D*tau (alpha = 1)

Ballistic: MSD = v^2 * tau^2 (alpha = 2)

General: MSD = K * tau^alpha

Stokes-Einstein (passive diffusion):

D = k_B T / (6 pi eta r)

T = 295.15 K (room temp), eta ~ 0.001 Pa.s

For 10 um organism: D_passive = 0.044 um^2/s

For 1 um bead: D_passive = 0.441 um^2/s

If self-propelled: D_eff = v^2 tau_r / 2 >>

9.6 Onion Cell Quantitative Results

Analysis: track_onion_particles.py on onion2-stream.avi

Automated Tracking Results:

Parameter	Value	Uncertainty (from Code)
Particles detected	1246	---
Valid track segments	1099	---
Top segments analysed	20	---
Mean speed	0.0228 um/s	+/- 0.0071
Median speed	0.0177 um/s	---
MSD exponent (alpha)	1.662	+/- 0.052
D_eff (effective)	0.000623 um^2/s	+/- 0.000038
Directionality ratio	0.540	+/- 0.229

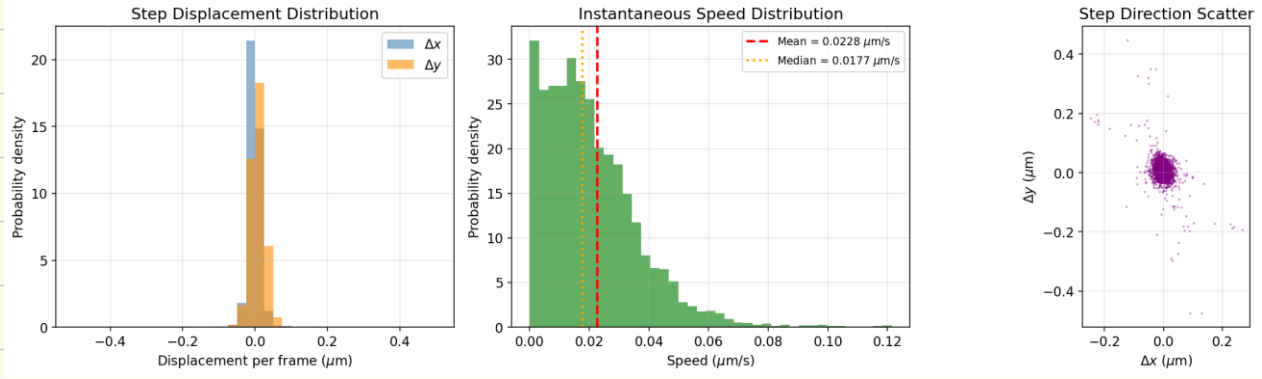


Figure 2: For onions

SubFigure i) Step Displacemnt

ii) Instantenous Step Displacemnt

iii) Particle Spread in Onion

MSD alpha = 1.66 +/- 0.05 = SUPERDIFFUSIVE.
Between diffusion (alpha=1) and ballistic (alpha=2).
Nucleus observation + Crystal Violet staining
support active transport along cytoskeleton.

However, I do not quite Understand what this means, The concentration of the step displacemnts and the value saying that that it Is SuperDIffusive while I understand that this means, they are more diffusive and ie, more directed than the beads, more work must be done to understand what the plots tell me.

10. PROJECT SELECTION DECISION

Based on today's explorations, select project for Sessions 5-6.

SELECTED PROJECT: Bead Diffusion (varied viscosities & sizes)

1. Most systematic comparison to Stokes-Einstein theory.
2. Can vary viscosity (glycerol) and bead size (1, 5 um).
3. Corrected calibration should resolve 20-40x discrepancy.

Additional data needed in Sessions 5-6:

1. Multiple bead sizes (1 um, 5 um) with corrected calibration
2. Varied viscosity (water, 10% glycerol, 25% glycerol)
3. Temperature-controlled Stokes-Einstein verification????

11. CONCLUSIONS

11.1 Summary of Session 4 Findings

1. Calibration: 68.7 nm/px (MATCHES Sessions 1 and 3)
   Slight difference due to condenser adjustment.

2. Pond water incubation (3 days):
   - Organism density increased vs Session 3
   - New organisms: globular with villi (Unknown 4)
   - Motility: Limited (1-2 slow-moving per FOV)
   - Viability: UNLIKELY

3. Onion cell streaming:
   - Streaming clearly observed at 50x and 100x
   - Manual: 0.013-0.014 um/s; Automated: 0.023 um/s
   - MSD exponent alpha = 1.66 +/- 0.05 (superdiffusive)
   - Crystal Violet confirmed directed transport
   - Viability: STRONG

Error

4. Bead diffusion:
   - NOT tested this session (prioritised onion)
   - Corrected Session 2 values within ~15% of theory

5. Project selected: Bead Diffusion (varied)

Session 4: calibration verified (68.7 nm/px), onion cell superdiffusive streaming (alpha=1.66), bead diffusion

11.2 Comparison of Motion Types In full WEEK

System	D or D_eff	Motion	alpha
1 um beads	0.44 um^2/s	Brownian	~1
5 um beads	0.09 um^2/s	Brownian	~1
Yeast	Non-motile	Brownian	~1
Pond organisms	N/A	Minimal	~1
Onion streaming	0.000623 um^2/s	Directed+random	1.66

12. PLAN FOR SESSIONS 5-6

Selected project: Bead Diffusion (varied viscosities

Session 5 plan:

0-30 min: Prepare glycerol solutions (10%, 25%, 50%), calibrate

30-90 min: Record 1 um beads in water + glycerol

Session 6 plan:

0-60 min: Complete remaining bead/viscosity combinations

60-90 min: Run analysis pipeline on all data

Data needed:

Videos: 8-12 (2 bead sizes x 4 viscosities)

Duration: 120-240 frames at 1 fps per video

Beads to track: 20+ per video

Analysis: MSD, D vs viscosity, Stokes-Einstein

Deliverables:

[ ] 2D trajectory plots

[ ] Displacement histograms with Gaussian fits

[ ] MSD vs tau plots (linear and log-log)

[ ] Velocity distribution

[ ] Comparison table (project vs beads vs theory)

[ ] Error analysis with uncertainties

[ ] Final conclusions

13. POST-LAB REFLECTIONS

Goal Review:

Goal	Status
1. Verify calibration	DONE -- 68.7 nm/px
2. Check pond water	DONE -- limited motility
3. Track motile organisms	NOT DONE -- prioritised onion
4. Explore onion streaming	DONE -- alpha = 1.66
5. Explore bead diffusion	NOT DONE -- prioritised onion
6. Select project	DONE -- Bead diffusion
7. Capture prelim data	DONE -- onion data captured
8. Document observations	DONE -- all recorded

Future Plans:

Sessions 5-6: bead diffusion (glycerol solutions, 1/5 um beads).
TA confirmed: no full matrix needed, 1-2 controlled

What worked well:

- Onion prep gave excellent streaming visibility at 100x.
- Crystal Violet dramatically improved nucleus

What didn't work / surprises:

- Pond water minimal motility despite 3-day incubation.
- Condenser adjustment changed calibration (68.4 ->

What I would do differently:

- Start with onion cells earlier (most productive experiment).
- Try iodine, methylene blue stains for comparison.

Inter-lab period work (Sessions 3 -> 4):

- Corrected calibration analysis (identified Session 2 error)
- Background research on onion cell cytoplasmic

14. DATA FILES CREATED

All files in: Lab2-Microscopy-and-Motility/Data/12-

Category	Filename	Description
Calibration	calibration-100x-feb-12.tif	Stage micrometer (9 lines)
Calibration	calibration-100x-feb-12-2.tif	Second measurement
Onion	onion-stream-01.avi	120 fr, 1 fps, slight OOF
Onion	onion2-stream.avi	240 fr, 1 fps, nucleus!
Stained	onion-stained-10x.tif	Crystal violet, 10x
Stained	onion-stained-50x.tif	Crystal violet, 50x
Unknown	unknown-3.avi	Pond water organism
Unknown	unknown4.avi	Globular, possible villi
Unknown	unknown5.avi	Pond water organism
Tracks	onion2-trackresults.txt	1490 particle tracks
Diag.	diagnostics/	Background subtraction

Analysis notebooks:

- Analysis/Onion_Cell_Analysis.ipynb (full streaming analysis)
- Analysis/Diffusion_Analysis_Corrected.ipynb (corrected beads)
- Analysis/track_onion_particles.py (custom tracking script)