PHYS 332 Advanced Physics Laboratory

Laboratory Notebook

Experiment: Microscopy and Cell Motility
Lab Period 2: Bead Size Comparison & Cell Imaging

Date	February 10, 2026
Time	1:30 PM - 4:30 PM
Lab Partners	Nathan (Author), Ahi
Workstation	Biophysics Lab, Station 2
Microscope	Carl Zeiss Axioskop 2 Plus
Camera	FLIR BlackFly S (1288 × 964 pixels)

Session Goals

The specific, measurable goals for this lab session are:

1. Review and verify completed analysis of 1 μm beads from Lab Period 1
2. Prepare samples of larger beads (2 μm and 3 μm diameter)
3. Capture 60-second videos of Brownian motion for each bead size
4. Extract trajectories and compute diffusion coefficients for all bead sizes
5. Verify $D \propto 1/r$ relationship (Stokes-Einstein) quantitatively
6. Qualitatively examine HT1080 fixed cells and yeast cells; estimate cell sizes

Pre-Lab Preparation

Completed: February 9, 2026 (day before lab)

Summary of Lab Period 1 Results

Key results from 1 μm bead analysis:

Parameter	Value
Calibration factor	97.7 ± 0.5 nm/pixel
Bead diameter	1.0 ± 0.05 μm
Temperature	22.3 ± 0.5°C
D (measured, MSD method)	0.465 ± 0.008 μm²/s
D (measured, variance method)	0.468 ± 0.008 μm²/s
D (Stokes-Einstein theory)	0.456 ± 0.015 μm²/s

Agreement with theory	Within 0.5σ (2% discrepancy)

Verification completed:
- Displacement histogram shows Gaussian distribution with mean ≈ 0
- MSD scales linearly with time (slope gives D)
- Log-log plot of MSD vs τ shows slope = 1 (diffusive behavior)
- Camera exposure correction (Eq. 3) applied: ~5% effect

Theoretical Predictions for Larger Beads

Using the Stokes-Einstein relation $D = k_B T / (6\pi\eta r)$, we predict:

Bead Diameter (μm)	Radius r (μm)	D predicted (μm²/s)	RMS displacement per frame
1.0	0.50	0.456	0.17 μm = 1.7 px
2.0	1.00	0.228	0.12 μm = 1.2 px
3.0	1.50	0.152	0.10 μm = 1.0 px

Expected scaling relationship:
$D(2\ \mu m) / D(1\ \mu m) = r(1\ \mu m) / r(2\ \mu m) = 0.5 / 1.0 = 0.5$
$D(3\ \mu m) / D(1\ \mu m) = r(1\ \mu m) / r(3\ \mu m) = 0.5 / 1.5 = 0.33$

CONCLUSION: Larger beads diffuse more slowly. Expect D(2μm) ≈ 0.23 μm²/s and D(3μm) ≈ 0.15 μm²/s at T = 22.3°C.

Dilution Calculations for Larger Beads

Target: ~20 beads in field of view (126 μm × 94 μm × 5 μm depth)
$V_{obs} = 5.92 \times 10^{-8}$ mL

For 2 μm beads:
$V_{bead} = (4/3)\pi(1\ \mu m)^3 = 4.19 \times 10^{-12}$ mL
$m_{bead} = 4.40 \times 10^{-12}$ g
Required number density: $n = 20 / 5.92 \times 10^{-8}$ mL $= 3.38 \times 10^{8}$ beads/mL
$c_{required} = 1.49 \times 10^{-3}$ g/mL
Dilution factor from 0.5 wt% stock: 5 mg/mL / 1.49 mg/mL ≈ 3.4
Dilution: 1:3 (10 μL stock + 20 μL water)

For 3 μm beads:
$V_{bead} = (4/3)\pi(1.5\ \mu m)^3 = 1.41 \times 10^{-11}$ mL
$m_{bead} = 1.49 \times 10^{-11}$ g
Required number density: $n = 20 / 5.92 \times 10^{-8}$ mL $= 3.38 \times 10^{8}$ beads/mL
$c_{required} = 5.02 \times 10^{-3}$ g/mL
Stock is already near target concentration.
Dilution: Use undiluted or slight 1:1 dilution if too dense

CONCLUSION: Dilution factors: 2 µm beads - 1:3 dilution; 3 µm beads - use undiluted or 1:1.

Experimental Work

Session Start: 1:30 PM

1. Microscope Setup and Calibration Verification

Time: 1:30 PM - 1:45 PM

Procedure:

7. Performed Köhler illumination setup (same as Lab Period 1)
8. Verified calibration with stage micrometer - confirmed 97.7 nm/pixel
9. Recorded room temperature: 22.1 ± 0.5°C (slightly cooler than Lab 1)

Variables Definition:

Variable Type	Variable	Description
Independent	Bead diameter d	1 µm, 2 µm, 3 µm
Independent	Time t	Frame number × frame interval
Dependent	D	Measured diffusion coefficient
Dependent	x(t), y(t)	Bead centroid position
Control	Temperature	22.1 ± 0.5°C
Control	Medium	Deionized water
Control	Frame rate	30 fps

2. Sample Preparation - Larger Beads

Time: 1:45 PM - 2:15 PM

2 µm beads (Polysciences Inc., Cat. #07304):

10. Vortexed stock for 30 seconds
11. Prepared 1:3 dilution: 10 µL stock + 20 µL DI water
12. Made thick chamber with Parafilm spacers
13. Loaded 10 µL sample, sealed with nail polish

Initial check: ~25 beads in FOV at 100× - good density

3 µm beads (Polysciences Inc., Cat. #17134):

14. Vortexed stock for 30 seconds
15. Prepared 1:2 dilution: 15 µL stock + 15 µL DI water (stock was dense)
16. Made thick chamber with Parafilm spacers
17. Loaded 10 µL sample, sealed with nail polish

Initial check: ~18 beads in FOV at 100× - acceptable density

Observation notes:

- 3 μm beads are clearly larger and more visible than 1 μm beads
- Motion of 3 μm beads appears slower (as expected)
- Some beads near bottom of chamber - focused on mid-plane

3. Data Collection - Brownian Motion Videos

Time: 2:15 PM - 3:00 PM

Camera settings (same for all):

Parameter	Value
Frame rate	30 fps
Exposure time	5 ms
Recording duration	60 seconds (1800 frames)
File format	Uncompressed AVI

Data files recorded:

Bead Size	Filename	Notes
2 μm	Data/2026-02-10/brownian_2um_trial1.avi	1800 frames, good
2 μm	Data/2026-02-10/brownian_2um_trial2.avi	Backup recording
3 μm	Data/2026-02-10/brownian_3um_trial1.avi	1800 frames, good
3 μm	Data/2026-02-10/brownian_3um_trial2.avi	Backup recording

Qualitative observations during recording:

- 2 μm beads: Clearly visible motion, but slower than 1 μm beads
- 3 μm beads: Very slow motion, beads barely move frame-to-frame
- No significant drift detected (chamber properly sealed)
- Temperature remained stable at 22.1°C throughout recording

CONCLUSION: Captured 60 s videos for 2 μm and 3 μm beads. Files saved to Data/2026-02-10/. Qualitatively, larger beads show slower Brownian motion.

4. Particle Tracking

Time: 3:00 PM - 3:30 PM

Method: ImageJ MTrack2 Plugin

Tracking parameters (adjusted for larger beads):

Parameter	2 μm beads	3 μm beads
Min object size	150 pixels²	300 pixels²
Max object size	800 pixels²	1200 pixels²
Max velocity	30 pixels/frame	20 pixels/frame

Min track length	500 frames	500 frames

Tracking results:

	2 μm beads	**3 μm beads**
Beads tracked	15	12
Average track length	1650 frames	1580 frames
Beads rejected	8	5
Output file	trajectories_2um.csv	trajectories_3um.csv

[Data analysis to be completed using brownian_motion_analysis.py]
Note: The Python analysis script generates all quantitative results, plots, and comparisons. Run the script with the trajectory CSV files to complete this section.

5. Expected Analysis Results (Placeholder)

Time: Analysis to be completed after lab

The following results will be generated by the Python analysis script:

[TABLE: Displacement Statistics - To be filled from script output]

Expected format:

Bead Size	**σ(Δx) (μm)**	**σ(Δy) (μm)**	**Mean (μm)**
1 μm	[from Lab 1: 0.178]	[0.175]	[≈0]
2 μm	[to be measured]	[to be measured]	[expected ≈0]
3 μm	[to be measured]	[to be measured]	[expected ≈0]

[TABLE: Diffusion Coefficient Comparison - To be filled from script output]

Expected format:

Bead Size	**D measured (μm²/s)**	**D theory (μm²/s)**	**Discrepancy**	**D ratio to 1μm**
1 μm	0.465 ± 0.008	0.456	+2%	1.00
2 μm	[to measure]	0.228	[to calc]	[expect ~0.5]
3 μm	[to measure]	0.152	[to calc]	[expect ~0.33]

[PLOT REFERENCES: The following plots will be generated by the analysis script]

- trajectory_2um.pdf - Example trajectory for 2 μm beads
- trajectory_3um.pdf - Example trajectory for 3 μm beads
- histogram_2um.pdf - Displacement histogram for 2 μm beads
- histogram_3um.pdf - Displacement histogram for 3 μm beads
- msd_comparison.pdf - MSD vs τ for all bead sizes (key verification plot)

- D_vs_inverse_radius.pdf - D vs 1/r to verify Stokes-Einstein

CONCLUSION: [Placeholder] Expected: D ∝ 1/r verified. D(2μm)/D(1μm) ≈ 0.5, D(3μm)/D(1μm) ≈ 0.33, consistent with Stokes-Einstein relation.

6. Qualitative Cell Imaging

Time: 3:30 PM - 4:15 PM

6.1 Yeast Cells (Saccharomyces cerevisiae)

Sample preparation:

18. Added 10 mL warm water (37°C) to 15 mL Falcon tube
19. Added ~2 g sugar and ~2 g baker's yeast
20. Stirred gently, let sit for 5 minutes until bubbling
21. Prepared thin wet mount (no spacer) with 5 μL sample

Observations (40× objective):

- Cells clearly visible as oval/ellipsoid shapes
- Many cells appear to be budding (cell division)
- Cells show some Brownian motion but mostly stationary
- Internal structure visible (darker regions = vacuoles?)

Size measurements:
Used ImageJ line tool on captured images

Cell #	Length (μm)	Width (μm)	Notes
1	5.8	4.2	Single cell
2	6.1	4.5	Single cell
3	8.5	4.8	Budding pair
4	5.5	4.0	Single cell
5	5.9	4.3	Single cell

Average size:
Single cells: 5.8 ± 0.3 μm × 4.2 ± 0.2 μm (ellipsoidal)
Literature value: 5-10 μm diameter - consistent with observations

Image saved:
Data/2026-02-10/yeast_cells_40x.tif

6.2 HT1080 Cells (Fixed Human Fibrosarcoma)

Sample:
Pre-prepared fixed mount (stained blue for contrast)

Observations (40× and 100× objectives):

- Cells are MUCH larger than bacteria or yeast
- Irregular, flattened shapes (adherent epithelial morphology)
- Nucleus clearly visible as darker blue region
- Cytoplasm extends with irregular projections

- Some cells appear to be dividing (mitotic figures)

Size measurements (40× objective):

Cell #	Length (μm)	Width (μm)	Notes
1	45	28	Spread cell
2	52	35	Large, irregular
3	38	22	Elongated
4	42	30	Rounded
5	48	32	Spread cell

Average size:
HT1080 cells: 45 ± 5 μm × 30 ± 5 μm
Nucleus diameter: ~12-15 μm
Literature: HT1080 cells typically 30-60 μm when spread - consistent

Image saved:
Data/2026-02-10/HT1080_cells_40x.tif
Data/2026-02-10/HT1080_cells_100x.tif

6.3 Comparison of Cell Types

Feature	E. coli (Lab 3)	Yeast	HT1080
Type	Prokaryote	Eukaryote	Eukaryote
Size	~2 × 0.5 μm	~6 × 4 μm	~45 × 30 μm
Shape	Rod	Oval	Irregular/spread
Nucleus	None	Yes	Yes (large)
Motility	Swimming	Minimal	None (fixed)

CONCLUSION: Cell sizes span 2 orders of magnitude: E. coli ~1 μm, Yeast ~5 μm, HT1080 ~45 μm. Morphology reflects function: bacteria are small/motile, yeast are simple eukaryotes, mammalian cells are large with complex structure.

Session End Summary

Time: 4:25 PM

Goals Evaluation

Goal	Status
Review Lab 1 analysis	✓ Complete
Prepare 2 μm and 3 μm bead samples	✓ Complete

Capture 60 s videos for each size	✓ Complete
Extract trajectories	✓ Complete
Compute D and verify D ∝ 1/r	⏳ Analysis pending
Image yeast and HT1080 cells	✓ Complete

Data Files Summary

File	Location
2 µm bead video	Data/2026-02-10/brownian_2um_trial1.avi
3 µm bead video	Data/2026-02-10/brownian_3um_trial1.avi
2 µm trajectories	Analysis/2026-02-10/trajectories_2um.csv
3 µm trajectories	Analysis/2026-02-10/trajectories_3um.csv
Yeast image	Data/2026-02-10/yeast_cells_40x.tif
HT1080 images	Data/2026-02-10/HT1080_cells_*.tif
Analysis script	Analysis/brownian_motion_analysis.py

Analysis Script Instructions

To complete the quantitative analysis, run the Python script:

22. 1. Update CONFIG section with correct file paths
23. 2. Verify calibration factor (97.7 nm/pixel) and temperature (22.1°C)
24. 3. Run: python brownian_motion_analysis.py
25. 4. Script will generate all plots and summary statistics
26. 5. Copy key results into this notebook

Preparation for Lab Period 3

- Answer Pre-lab Questions 6-8 (bacterial motion)
- Calculate E. coli dilution factor from OD600 = 1.0
- Review run-and-tumble motion of wild-type bacteria
- Compare expected motion: mutant (smooth swimming) vs wild-type (run-and-tumble)
- Complete full analysis of bead data and generate plots

Reflections

The qualitative observations clearly show that larger beads diffuse more slowly, consistent with Stokes-Einstein. The cell imaging provided excellent comparison of prokaryotic vs eukaryotic cell sizes and morphologies. Key points:

- Tracking parameters must be adjusted for different bead sizes
- 3 µm beads move very slowly - need long recordings for good statistics
- Yeast cells are ~10× larger than E. coli, HT1080 cells are ~10× larger than yeast
- Fixed cells (HT1080) allow detailed imaging but no motility studies

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Submitted: February 10, 2026

Week 2 Submission Summary

(Required for snapshot submission)

This summary provides page references to key items completed during Lab Period 2:

Item	Page Reference
Session goals and variables definition	Pages 1-2
Lab Period 1 results summary	Page 3
Theoretical predictions for larger beads	Page 3
Dilution calculations	Pages 3-4
Sample preparation procedure	Page 5
Data collection details	Pages 5-6
Particle tracking parameters	Page 7
Analysis placeholder (pending script)	Page 7
Yeast cell observations and measurements	Page 8
HT1080 cell observations and measurements	Pages 8-9
Cell type comparison table	Page 9
Goals evaluation	Page 10
Data files summary	Page 10

Key boxed conclusions:
- Page 3: Expected D values for 2 μm and 3 μm beads
- Page 4: Dilution factors calculated
- Page 6: Data collection complete
- Page 7: $D \propto 1/r$ verification pending
- Page 9: Cell size comparison (2 orders of magnitude)

Inter-lab period work:
- Pre-lab calculations for larger beads (Page 3-4)
- Python analysis script development (see attached)
- Review of Lab Period 1 results (Page 3)

Attachments:
- brownian_motion_analysis.py - Complete Python analysis script
- Analysis plots (to be generated after running script)