

# Lab Notebook Submission Summaries

Microscopy and Cell Motility (Lab 2) – Sessions 3 & 4

Ahilan Kumaresan & Nathan Unhrn  
PHYS 332

## Week 3 Submission: Session 3

Files: <https://github.com/Ahilan-Bucket/phys332W-sfu/tree/main/Lab2-Microscopy-and-Motility>

### Session Overview

**Date:** 10-Feb-2026

**Lab Partner:** Nathan Unhrn

**Pages:** 6–26

**Recorder:** Ahilan Kumaresan

**Objective:** Observe live cells (yeast, HT1080 stained slides, pond water organisms); pivot from *E. coli* plan after culture became unavailable; recalibrate microscope; collect preliminary data on motile organisms.

| Item  | Page  |
|---|-------|
| <b><i>Preparation</i></b>   |       |
| Goals (revised after <i>E. coli</i> unavailable), Variables, Apparatus                | 6     |
| <b><i>Calibration</i></b>   |       |
| Calibration re-verification (100×): 68.4 nm/px — matches Session 1                    | 8     |
| <b><i>Pond Water</i></b>  |       |
| SFU campus pond water collection procedure  | 10    |
| Wet mount preparation; comparison of chamber types (spacer vs no spacer)              | 11    |
| General observations: cells and debris present, no motility                           | 11    |
| <b><i>Unknown Cells</i></b>   |       |
| Unknown Cell 1: ~55–58 µm, possible <i>Arcella</i> or air bubble/spore; Image 1       | 13    |
| Unknown Cell 2: ~14 × 19 µm, possibly <i>Pandorina</i> (colonial green alga); Image 2 | 15    |
| <b><i>Yeast</i></b>   |       |
| Yeast imaging: 3 dilutions (1:10, 1:100, 1:200); Brownian vibration only              | 17    |
| Sugar gradient experiment idea for future sessions                                    | 19    |
| <b><i>HT1080</i></b>  |       |
| HT1080 malignant cells (pre-stained, 40×): irregular web-like morphology; Image 4     | 19    |
| <b><i>Conclusions &amp; Reflection</i></b>  |       |
| Conclusions: calibration confirmed, no motile cells found, deferred to Session 4      | 21    |
| Plan for Session 4: incubated pond water, onion streaming, bead diffusion             | 23    |
| Post-lab reflections; data files summary  | 25–26 |

**Key Files:**

- `calibrate0000.tif`, `calibrate0001.tif` — Calibration images
- `yeast-10x.avi`, `yeast-100x.avi`, `yeast-200x.avi` — Yeast dilution videos
- `unknown.tif`, `unknown2.tif` — Unknown pond water organisms
- `ht1080.tif` — HT1080 malignant cells

**Key Measurements (Session 3):**

| Parameter         | Value       | Notes                           |
|-------------------|-------------|---------------------------------|
| Pixel size (100×) | 68.4 nm/px  | Matches Session 1 (68.45 nm/px) |
| Unknown Cell 1    | ~55–58 µm   | Possible spore or air bubble    |
| Unknown Cell 2    | ~14 × 19 µm | Possibly <i>Pandorina</i>       |

## Week 4 Submission: Session 4

### Session Overview

**Date:** 12-Feb-2026

**Lab Partner:** Nathan Unhrn

**Pages:** 52–81

**Recorder:** Ahilan Kumaresan

**Objective:** Verify calibration; check incubated pond water for motile organisms; explore onion cell cytoplasmic streaming as candidate short project; select project for Sessions 5–6.

| Item  | Page  |
|---|-------|
| <b><i>Preparation</i></b>   |       |
| Goals (8 objectives), Apparatus, Variables, References  | 52–55 |
| Microscope setup verification: Köhler illumination, calibration   | 56    |
| <b><i>Calibration</i></b>   |       |
| Calibration verification: 68.7 nm/px — matches Sessions 1 & 3   | 56    |
| Calibration error history: Session 2 (345 nm/px) was WRONG; correction factor 25.4×                         | 58    |
| Corrected $D$ values: 1 $\mu\text{m}$ beads within $\sim 15\%$ of Stokes–Einstein                           | 58    |
| <b><i>Pond Water Incubation Check</i></b>   |       |
| Visual inspection of 3-day incubated water; new collection technique  | 60    |
| Microscope observations: more organisms, limited motility   | 60    |
| New organism 3 & 4 identification; Image 1, Image 2   | 62    |
| <i>Conclusion: collection method &gt; incubation time; pond water unlikely as project</i>                   |       |
| <b><i>Onion Cell Exploration</i></b>  |       |
| Background: cytoplasmic streaming (cyclosis), actin/myosin transport  | 64    |
| Onion preparation; observations at 50 $\times$ and 100 $\times$ ; Image 4                                   | 64–65 |
| Video capture: <code>onion-stream-01</code> (120 fr), <code>onion2-stream</code> (240 fr, nucleus visible!) | 66    |
| Manual velocity: Granule 1 = 0.0125 $\mu\text{m/s}$ , Granule 2 = 0.0143 $\mu\text{m/s}$                    | 66    |
| Crystal Violet staining: nucleus and cell structures visible  | 68    |
| <i>Key observation: “We are seeing the nucleus! Lots of material going towards it!!”</i>                    |       |
| <b><i>Post-Lab Analysis</i></b>   |       |
| Qualitative motion comparison table (beads vs yeast vs pond vs onion)                                       | 69    |
| Automated trajectory analysis: custom Python tracking pipeline; Figure 1                                    | 69–70 |
| MSD log–log analysis: $\alpha = 1.662 \pm 0.052$ (superdiffusive); Figure 2                                 | 71    |
| Velocity estimates table (manual + automated)   | 71    |
| Quantitative results: 1246 particles, 20 top segments; Table + Figures A–C                                  | 73–74 |
| <b><i>Project Selection &amp; Conclusions</i></b>   |       |
| Decision matrix: Bead Diffusion selected (score 15 vs Onion 13, Pond 9)                                     | 75    |
| Conclusions: calibration verified, onion $\alpha = 1.66$ , bead diffusion for Sessions 5–6                  | 76    |
| Plan for Sessions 5–6: glycerol solutions, bead sizes, Stokes–Einstein verification                         | 78    |
| Post-lab reflections; data files inventory  | 79–81 |

**Key Files:**

- `calibration-100x-feb-12.tif` — Calibration image
- `onion-stream-01.avi` — Onion streaming video (120 frames)

- `onion2-stream.avi` — Onion streaming video (240 frames, nucleus visible)
- `onion-stained-10x.tif`, `onion-stained-50x.tif` — Crystal Violet stained onion
- `unknown-3.avi`, `unknown4.avi` — New pond water organisms
- `onion2-trackresults.txt` — Tracking data export

#### Key Measurements (Session 4):

| Parameter                             | Value  | Notes                             |
|---------------------------------------|--|-----------------------------------|
| Pixel size (100×)                     | 68.7 nm/px                                       | Confirmed (condenser shift ~0.4%) |
| Mean streaming speed                  | $0.0228 \pm 0.0071$ $\mu\text{m/s}$              | Automated tracking (top 20)       |
| MSD exponent $\alpha$                 | $1.662 \pm 0.052$                                | Superdiffusive                    |
| $D_{\text{eff}}$ (onion)              | $0.000623 \pm 0.000038$ $\mu\text{m}^2/\text{s}$ | Effective diffusion               |
| Directionality ratio                  | $0.540 \pm 0.229$                                | Mixed directed + random           |
| Corrected $D$ (1 $\mu\text{m}$ beads) | $0.507$ $\mu\text{m}^2/\text{s}$                 | Within ~15% of theory (0.441)     |

## Executive Summary

### Key Figures

| Figure       | Description  | Page |
|--------------|--|------|
| Image 1 (S3) | Unknown cell 1 from SFU pond water ( $\sim 55\text{--}58\ \mu\text{m}$ ) | 15   |
| Image 2 (S3) | Unknown cell 2 from SFU pond water ( $\sim 14 \times 19\ \mu\text{m}$ )  | 16   |
| Image 4 (S3) | HT1080 malignant cells at $100\times$                                    | 21   |
| Image 1 (S4) | Unknown organism 3 from incubated pond water                             | 62   |
| Image 2 (S4) | Unknown organism 4 at $100\times$  | 62   |
| Image 4 (S4) | Onion epidermis cells at $100\times$ , granules visible                  | 65   |
| Figure 1     | 2D trajectories of onion granules  | 70   |
| Figure 2     | MSD log–log plot, $\alpha = 1.66$  | 71   |
| Figure A     | Onion cell particle trajectories (quantitative)                          | 74   |
| Figure B     | Displacement statistics  | 74   |
| Figure C     | MSD analysis log–log plot  | 74   |

### Key Tables

| Table                   | Description  | Page |
|-------------------------|--|------|
| Calibration history     | Sessions 1–4 pixel size comparison                             | 58   |
| Corrected $D$ values    | $1\ \mu\text{m}$ and $5\ \mu\text{m}$ beads vs Stokes–Einstein | 58   |
| Pond water comparison   | Session 3 vs Session 4 incubation changes                      | 60   |
| $100\times$ observation | Onion cell features at high magnification                      | 65   |
| Video recording log     | Two onion streaming recordings                                 | 66   |
| Motion comparison       | Beads vs yeast vs pond vs onion (qualitative)                  | 69   |
| Automated tracking      | 8 parameters with uncertainties                                | 73   |
| Decision matrix         | Project selection scores (weighted)                            | 75   |
| Motion types            | $D$ , motion type, $\alpha$ for all systems                    | 76   |

## Conclusions

### Calibration (p. 58):

Session 2 used  $0.345\ \mu\text{m}/\text{px}$  (INCORRECT). Correct:  $0.0684\ \mu\text{m}/\text{px}$ , confirmed in Sessions 1, 3, and 4.

This resolves the  $20\text{--}40\times$  discrepancy in Week 1  $D$  values.

Corrected  $1\ \mu\text{m}$  bead  $D$  within  $\sim 15\%$  of theory.

### Onion Cell Streaming (p. 71):

MSD exponent  $\alpha = 1.662 \pm 0.052 \rightarrow$  **superdiffusive**.

Between pure diffusion ( $\alpha = 1$ ) and ballistic ( $\alpha = 2$ ).

Consistent with directed cytoplasmic streaming along actin filaments + random thermal fluctuations.

### Project Selection (p. 75):

**Bead Diffusion** selected for Sessions 5–6.

Best systematic comparison to Stokes–Einstein theory  
with varied viscosity (glycerol) and bead size (1, 5  $\mu\text{m}$ ).

**Summary**

Sessions 3–4 transitioned from passive observations (beads, fixed cells) to exploring active biological transport. The key discovery was **onion cell cytoplasmic streaming** with MSD exponent  $\alpha = 1.66 \pm 0.05$ , clearly distinguishing directed intracellular transport from Brownian diffusion. The calibration error from Session 2 ( $5\times$  overestimate) was definitively resolved by consistent measurements across Sessions 1, 3, and 4 (all within 68.4–68.7 nm/px).

**Not completed:** *E. coli* imaging (culture unavailable), bead diffusion verification (deferred to Sessions 5–6).

**Selected project for Sessions 5–6:** Bead Diffusion (varied viscosities and sizes) + yeast cell population study.

---